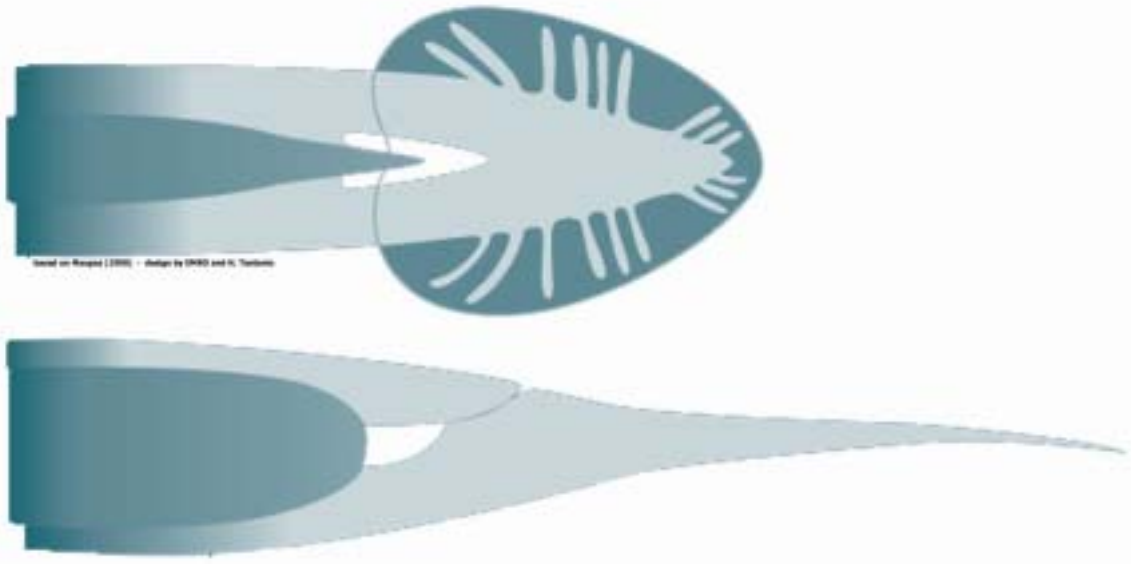
ON THE STUDY OF EVOLUTIONARY BIOLOGY WITH **CAENORHABDITIS ELEGANS** AND RELATED SPECIES

EMBO WORKSHOP
INSTITUTO GULBENKIAN DE CIENCIA
MAY 2006 OEIRAS PORTUGAL

FUNDAÇÃO CALOUSTE GULBENKIAN

EMBO



PROGRAM

Tuesday, 23rd May

Afternoon Reception at the Hotel and host Institute (IGC)
19:00 Dinner at the host Institute (IGC)
20:00 Workshop info: Henrique Teotónio
20:10 **Patrick Phillips**: The study of evolutionary biology with *Caenorhabditis*

Wednesday, 24th May

9:00 **David Fitch** (moderator): Diversity and history of *Caenorhabditis*
9:15 **Karin Kiontke**: *Caenorhabditis* phylogeny and evolution
9:45 **Scott Baird**: Clade structure and gene flow in *Caenorhabditis briggsae*

10:15 Break

10:45 **Jody Hey** (moderator): The puzzles of *C. elegans* population genetics
11:00 **Antoine Barrière**: Population genetics and dynamics of *C. elegans*
11:30 **Asher Cutter**: Population genetic variation in *Caenorhabditis*: implications for demography and evolution

12:00 Lunch

13:00 Poster Session and Informal Meetings

17:00 **Dave Pilgrim** (moderator): Genetics of breeding system evolution
17:15 **Eric Haag**: Using comparative genetics to study the evolution of *Caenorhabditis* hermaphroditism
17:45 **Ronald Ellis**: Evolution of hermaphroditism
18:15 **King Chow**: Sex pheromone in nematode: developmental synthesis, perception and evolutionary implication

19:00 Dinner

Thursday, 25th May

9:00 **Kelley Thomas** (moderator): Mutation
9:15 **Dee Denver**: Mutation and genome evolution in *Caenorhabditis elegans*
9:45 **Andy Peters**: Evolution via compensatory mutations in *C. elegans*

10:15 Break

10:45 **Henrique Teotónio** (moderator): Genetic variation and experimental evolution
11:00 **Elie Dolgin**: Inbreeding and outbreeding depression in *Caenorhabditis*
11:30 **Matthew Salomon**: Understanding genetic variation in Rhabditid nematodes

12:00 Lunch

13:00 Poster Session and Informal Meetings

17:30 **Jan Kammenga** (moderator): Genetic analysis of complex phenotypes
17:45 **Matthew Rockman**: Finding the nucleotides underlying phenotypic variation in *C. elegans*
18:15 **Hinrich Schulenburg**: An evolutionary perspective in innate immunity and pathogen defense

19:00 Dinner

Friday, 26th May

9:00 **Ricardo Azevedo** (moderator): Evolution of development in *C. elegans*:
9:15 **Simon Harvey**: Natural variation in the plasticity of the development of dauer larvae: quantitative trait mapping and fitness consequences.
9:45 **Christian Braendle**: Robustness and evolution of *Caenorhabditis* vulval development

10:15 Break

10:45 **Scott Emmons** (moderator): Evolution of development in the *Caenorhabditis* genus
11:00 **Helen Chamberlin**: Evolution of excretory system features in *Caenorhabditis*
11:30 **Bhagwati Gupta**: Evolution of vulval development in *Caenorhabditis* nematodes

12:00 Lunch

13:00 Poster Session and Informal Meetings

15:45 **Marie-Anne Félix** (moderator): Genomics and Resources for the *Caenorhabditis* scientific community
16:00 **Avril Coghlan**: A program that predicts nematode genes by combining well conserved exons predicts by existing gene finders
16:30 **Erich Schwartz**: Cis-regulatory analysis of three *Caenorhabditis* genomes.
17:00 ***C. briggsae* genetics team**: Integrating genetic and genomic resources for *C. briggsae*
17:30 Group Discussion on common resources to the community and future perspectives.

18:30 Final Social Event and Dinner at the Palace of Marquês de Pombal

Saturday, 27th May

Trip to Sintra

TALK ABSTRACTS

TUESDAY, 23RD MAY

20:00 Workshop information

20:10 Evolutionary Biology with *Caenorhabditis*

Patrick Phillips
(pphil@uoregon.edu)

Address: Center for Ecology and Evolutionary Biology
5289 University of Oregon, Eugene, OR 97403-5289 USA

Abstract:The vast amount of genetic and functional data available for *C. elegans* has long recommended it as a useful system for addressing evolutionary questions. These efforts have been slowly gaining steam, and this workshop serves both as a celebration of the growth of this field and as an opportunity to set the agenda for what is clearly a new era in nematode evolutionary genetics. I will review existing community level resources available for genetic analysis and initiate the discussion of where our joint efforts might best lie. I will also review some major questions in nematode evolutionary biology and consider the possibility that different species might be best suited for particular sets of questions.

WEDNESDAY, 24TH MAY

9:00 The Diversity and History of *Caenorhabditis*

David Fitch
(david.fitch@nyu.edu)

Address: Department of Biology, New York University,
Main Building, Room 1009,
100 Washington Square East, New York, NY 10003, USA.

Abstract:*C. elegans* is a model animal and a model nematode, but how well it represents animals and nematodes depends on what features can be generalized from the model. The phylogenetic relationships within *Caenorhabditis,* between *C. elegans* and other nematodes and animals in general provide the necessary framework for these comparisons. Work in progress on the phylogenetic context for *Caenorhabditis* will be briefly reviewed.

9:15 *Caenorhabditis* phylogeny and evolution

Karin Kiontke
(kk52@nyu.edu)

Authors: Kiontke, K.[1], D. Fitch[1], and W. Sudhaus[2]
Address: [1] Department of Biology, New York University, New York, NY 10003, USA;
[2] Institut für Biologie/Zoologie, Freie Universität Berlin, 14195 Berlin, Germany

Abstract: *C. elegans* is widely regarded as *the* model nematode. This is not surprising, given the wealth of knowledge collected on this single species, including the complete genome sequence. However, comparative studies between *C. elegans* and other nematodes suggest many features of *C. elegans* and its close relatives are peculiar to this group. As a model "nematode", however, we would like to know which characteristics of

C. elegans are shared ancestrally with other nematodes. It is therefore important to know at which point of the nematode phylogeny the *C. elegans* characters evolved.

Here we review our efforts to delineate the position of *C. elegans* in the phylogenetic tree of Nematoda, and more specifically its relationships to other rhabditids and species within *Caenorhabditis*. We then evaluate *C. elegans* characters with respect to their distribution: are they nematode specific, specific for free living nematodes, specific for species in *Caenorhabditis*, or specific for *C. elegans* alone? Recent comparative studies provide information for a variety of character complexes: morphological characters, genetic and genomic characters (genome size and aspects of genome organization, intron number, susceptibility to RNAi), developmental and life history characters (development of vulva and male tail, reproductive mode, embryology), and ecological characters (habitat, phoresy, susceptibility to pathogens). In conclusion we follow the evolution of some interesting characters along the branches of the phylogenetic tree, and present *C. elegans* as a patchwork of ancestral (plesiomorphic) and newly derived (apomorphic) characters.

This research is supported by the Human Frontier Science Program and the National Science Foundation.

9:45 Clade structure and gene flow in *Caenorhabditis briggsae*

Scott Baird
(scott.baird@wright.edu)

Authors: Baird, S., and R. Hampton
Address: Department of Biological Sciences,
Wright State University, Dayton OH 45435-0001, USA

Abstract: Phylogenies of seventeen *C. briggsae* strains have been reconstructed from sequence comparisons at nine loci. Reconstructions made from a concatenated data set strongly support the division of *C. briggsae* into two distinct clades. This clade structure was supported by reconstructions made from seven of nine individual loci. At *Cb-her-1* shared polymorphisms were observed between clades, evidence of gene flow at this locus. Shared polymorphisms were not observed at other loci, suggesting that gene flow between clades is restricted in some regions of the genome but not others. Consistent with this, evidence for reproductive isolation between clades has been obtained from reciprocal crosses between strains AF16 and HK104. Approximately one third of F2 progeny from these crosses exhibited a delayed development phenotype and have a correspondingly reduced intrinsic growth rate. These phenotypes result from cryptic variation at maternal- and zygotic-effect genes. Zygotic effects have been linked to *Cb-glp-1* and *Cb-egl-5*.

10:15 Break

10:45 The puzzles of *C. elegans* population genetics

Jody Hey
(Hey@Biology.Rutgers.Edu)

Address: Rutgers University, Nelson Biological Labs
Piscataway, NJ 08854-8082, USA

Abstract: *Caenorhabditis elegans* presents some fascinating population genetic puzzles. Though *C. elegans* is globally distributed and not uncommon, it reveals patterns of low genetic variation suggestive of a small effective population size. This could be caused by very low outcrossing rates. Yet recent estimates of rates suggest that outcrossing is not so low that natural selection would drive down effective population size. Also patterns of codon usage suggest that natural selection is an effective force on very subtle fitness differences, consistent with a large effective population size. Another set of related puzzles concerns the relationship between local population structure, effective outcrossing rates, and linkage disequilibrium (LD). *C. elegans* strains collected from nature exhibit signs of significant population structure especially on the most local scales. This can reduce the rate of effective outcrossing, and contribute to LD over broader regional scales.

11:00 Population genetics and dynamics of *C. elegans*

Antoine Barrière
(barriere@ijm.jussieu.fr)

Authors: Barrière, A., and M.-A. Félix
Address: Institut Jacques Monod, Tour 43, 2 place Jussieu,
75251 Paris cedex 05, France

Abstract : *Caenorhabditis elegans* is a major model system in biology, yet very little is known about its biology outside the laboratory. Following our recent works on natural history, molecular diversity and outcrossing rates in wild populations , we pursued our samplings and followed C. elegans populations in several localities. Population density was recorded and sampled individuals were genotyped at 6 microsatellite loci. Our results show a maintenance of linkage disequilibrium over the span of several years and evidence of population persistence, and confirm the low outcrossing rate previously described.

11:30 Population genetic variation in *Caenorhabditis*: implications for demography and evolution

Asher Cutter
(asher.cutter@ed.ac.uk)

Address: Institute of Evolutionary Biology
University of Edinburgh, King's Buildings, W. Mains Rd.
Ashworth Labs, Room 123, Edinburgh, EH9 3JT, UK

Abstract: An understanding of the relative contributions of different evolutionary forces on an organism's genome requires an accurate description of the patterns of genetic variation within and between natural populations. To this end, I report a survey of nucleotide polymorphism for wild population samples of the nematodes *Caenorhabditis elegans*, *C. briggsae*, and *C. remanei*. The isolated strains derive principally from wild populations of several regions within France, Germany, Scotland, USA and China, in addition to stock center isolates. Both of the self-fertile species are characterized by low overall levels of genetic diversity, but only *C. briggsae* shows evidence of a correspondence between geography and patterns of variation. Linkage disequilibrium is evident, extending even between chromosomes, although recombination has played a role in the ancestry of extant polymorphism of both species. In stark contrast, the obligate outcrossing *C. remanei* exhibits approximately 20-fold greater diversity and linkage disequilibrium decays demonstrably over a few hundred base-pairs at most loci. I will discuss the patterns of polymorphism in relation to their implications for demography, selfing rate, molecular evolution, and the origins of the self-fertile reproductive mode.

12:00 Lunch

13:00 Poster Session and Informal Meetings

17:00 Genetics of Breeding System Evolution

Dave Pilgrim
(dave.pilgrim@ualberta.ca)

Authors: C.E. de Carvalho[1,3], P. Stothard[1], R.C. Hill[2],
D. Kelleher[2], E. Haag[2], and D. Pilgrim[1]
Address: [1]Univ. of Alberta, Edmonton, Alberta, Canada
[2]Univ. of Maryland, College Park, Maryland USA
[3]Harvard Medical School, Boston, Massachusetts USA

Abstract: *C. elegans* has proven to be an excellent genetic system to study signal transduction, pathways and we are developing a molecular picture of a signal transduction system involved in sex determination. Although sex is determined in *C. elegans* by the number of sex (**X**) chromosomes, mutations have been isolated which cause the animal to completely ignore the chromosomal signal. Two of the major classes of mutations are the Tra genes (in which both **XX** and **X0** animals develop as males) and the Fem genes (in which **XX** and **X0** develop as females). As well, several genes affect sexual development of the germline

(e.g. Fog and Mog genes). The molecular analysis of this pathway in *C. elegans* has been hindered by the finding that many of the proteins do not have clear sequence homologues in other systems, and cell biological function is not always easy to infer. Several labs have begun use of other *Caenorhabditis* species to aid in this characterization, under the assumption that sequence homologues of these genes in, say, *C. briggsae* will allow assignment of functional domains based on sequence conservation (or lack therof). Two surprises have arisen from this analysis: first, that the orthologues of the *C. elegans* sex determining proteins are diverging at a much faster rates than 'typical' protein species within the genus (and in at least one case are missing from *C. briggsae*); and second, that RNAi mediated gene knockdowns have produced phenotypes in other *Caenorhabditis* species that differ from expectations, raising the idea that the sex determination pathway is regulated differently in these different species. In particular, the evolution of a male/hermaphrodite reproductive mode (androdioecy) from a male/female (gonochoristic) ancestor appears to have occurs independently in *C. elegans* and *C. briggsae*. Different aspects of these studies will be discussed in detail by the other speakers in this session.

Our lab has been particularly interested in *fem-2*, which acts at an important branch point in the pathway for the regulation of sex determination in both the soma and germ line. FEM-2 is a protein phosphatase and is thought to signal between a cell surface receptor (TRA-2) and a nuclear transcription factor (TRA-1). We are examining the structure-function relationships for the (rapidly diverging) FEM-2 homologues in other species of *Caenorhabditis*. The conspecific interaction between the FEM-2 and FEM-3 proteins observed in *C. elegans* also occurs in *C. remanei*. To further explore whether the rapid evolution of FEM-2 and FEM-3 affects their molecular interactions, we tested for cross-species interactions between the proteins from *C. elegans*, *C. briggsae*, and *C. remanei*. Although all FEM-2/FEM-3 pairs from a single species interact, only two out of six interspecific pairs bind each other, showing that FEM-2 and FEM-3 are co-evolving. Both interspecific interactions involved C. *briggsae* FEM-3. We constructed chimeric versions of FEM-2 consisting of various combinations of the *C. elegans* and *C. remanei* proteins. *C. briggsae* FEM-3 interacted with all the chimeras, even those that did not interact with either *C. elegans* or *C. remanei* FEM-3. We hypothesize that the promiscuity of *C. briggsae* FEM-3 reflects an increased reliance on evolutionarily constrained regions of FEM-2 for binding. If so, our data support the notion that the co-evolution of two interacting proteins sometimes involves a shift in the domains that contribute to binding.

In collaboration with the Haag lab, we have undertaken genetic screens to test whether the observed sequence divergence between orthologues is underlying functional changes at the protein level. Although mutations in the *C. briggsae tra-1* and *tra-2* genes show grossly similar phenotypes in the two species, *C. briggsae* requires neither *fem-2* nor *fem-3* for hermaphrodite development, and XO *Cb-fem-2/3* animals are transformed into hermaphrodites, not females as in *C. elegans*. Exhaustive screens for suppressors of the *tra* mutations identified another 75 *fem*-like mutants, but all are self-fertile hermaphrodites rather than females. Control of hermaphrodite spermatogenesis therefore acts downstream of the *fem* genes in *C. briggsae.*

17:15 Using Comparative Genetics to Study the Evolution of *Caenorhabditis* Hermaphroditism

Eric Haag
(ehaag@umd.edu)

Authors: Hill, R.C., A.V. Doty, B. Schlager, E.S. Haag
Address: Department of Biology, University of Maryland
College Park, MD 20742, USA.

Abstract: The *Caenorhabditis* sex determination pathway is especially appealing for studying the evolution of development. It is well understood in *C. elegans*, its genes are some of the most rapidly evolving in the genome, and there is variation in reproductive strategies within the genus that depend upon its modification. Further, recent phylogenetic studies suggest *C. elegans* and *C. briggsae* may have evolved hermaphroditism independently, providing an excellent opportunity to study the developmental genetic details of convergent evolution. Comparative studies using RNA interference (RNAi) have revealed many similarities in sex determination across the genus, but also some intriguing differences. It is unclear if these results are a biological reality or a limitation of RNAi; true mutations are needed to clarify the results.

Our lab has used PCR-based deletion screens to identify mutants in *C. briggsae* sex determination genes, focusing on the *fem* class. The three *fem* genes are required for the development of all male cell types in *C. elegans*, including XX hermaphrodite sperm. In addition, *fem-3* must be negatively regulated to allow the switch to oogenesis. We generated null mutations in *Cb-fem-2* and *Cb-fem-3* and characterized their phenotypes. Interestingly, the characteristic self-sterile phenotype of XX *C. elegans fem-2* and *fem-3* mutants is not seen. Also, three independent lines of genetic evidence show that XO *Cb-fem-2* and *Cb-fem-3* mutants are transformed into hermaphrodites, and not into females as in *C. elegans*. These results indicate, surprisingly, that XX spermatogenesis is controlled downstream of the *Cb-fem* genes in *C. briggsae*. The

overtly similar hermaphrodites of *C. elegans* and *C. briggsae* are thus produced by intervention at different points in the conserved core sex determination pathway. Despite these differences, expression of *Cb-fem-2* and *Cb-fem-3* in the hermaphrodite germline is similar to that of *C. elegans*.

Our results to date strongly support the hypothesis of independent origins of self-fertility in *C. elegans* and *C. briggsae*. More recent screens have produced mutations with germ line-specific sexual transformation, which may affect the genes that act in *C. briggsae* to modulate the core pathway. We expect that these will be novel sex determination components or conserved players with novel activities

17:45 Evolution of hermaphroditism

Ronald Ellis
(ellisre@umdnj.edu)

Authors: Ellis, R.E., Y. Guo, and C. Baldi
Address: The UMDNJ School of Osteopathic Medicine
B303 Science Center, Stratford, NJ 08084, USA

Abstract: In some species of nematodes, *XX* animals become self-fertile hermaphrodites, but in other species they become females. Although hermaphrodites and females have similar bodies, the hermaphrodites make sperm early in life and oocytes during adulthood. Thus, we believe that understanding the control of germ cell fate is the key to learning how this mating system evolves.

Our phylogeny showed that *C. remanei* and *C. briggsae* are the most closely related species in this genus, even though one makes females and the other makes hermaphrodites. Furthermore, it implied that hermaphroditism might have evolved separately in *C. elegans* and *C. briggsae*. If so, underlying features of nematode sex-determination probably favor this change.

What are these features? Since *fog-1* and *fog-3* control which cells make sperm in *C. elegans*, we cloned their homologs from other nematodes. Their protein sequences have been diverging rapidly, but RNA interference shows that their functions are conserved. Each species requires *fog-1* and *fog-3* to make sperm, and animals make oocytes if either gene is targeted with RNAi.

Why do *fog-1* and *fog-3* cause *XX* animals to make sperm in some species, but not in others? We found that *fog-3* transcripts are expressed during larval development in *XX* hermaphrodites, but not in *XX* females. By contrast, *fog-1* is expressed more broadly. The expression of *fog-3* is controlled by upstream sex-determination genes, which are likely to act through conserved TRA-1A binding sites in the *fog-3* promoter. Knocking down *tra-1* messages with RNAi alters *fog-3* expression and alters germ cell fates.

We are now studying these upstream regulators. In *C. elegans, fog-2* mutations transform *XX* animals into true females. We identified a similar mutation, *glf-1(v35),* in *C. briggsae.* Genetic mapping and sequence analyses imply that this gene is a novel regulator of sexual fate. We are using SNP mapping to clone it, and have identified a tightly linked super-contig. We are also characterizing additional mutations that produce *XX* females. We conclude that a single mutation is sufficient to transform a hermaphroditic species into a female one.

In a reciprocal experiment, we used RNAi to produce *C. remanei XX* animals that make sperm and oocytes. Surprisingly, these 'pseudo-hermaphrodites' are not self-fertile. We know that their oocytes are normal, because they can be fertilized by sperm from males. However, their own sperm do not appear active, and do not track to the spermatheca, although they can be activated by pronase *in vitro,* and do stimulate ovulation *in vivo.* Based on these observations, and the existence of *C. elegans* mutations that block the activation of hermaphrodite sperm, we propose that the evolution of self-fertile hermaphrodites from females requires at least two steps. (1) The *XX* animals must acquire the ability to produce sperm and oocytes, and (2) the *XX* sperm must acquire the ability to self-activate, so they can fertilize the oocytes. Preliminary results suggest that male seminal fluid might be capable of activating pseudo-hermaphrodite sperm *in vivo,* although the efficiency appears to be low. Perhaps this mechanism allowed for the transition from females to hermaphrodites during evolution.

Finally, we suspect that the simple circuit of *tra-1, fog-1,* and *fog-3* we identified in nematodes allows rapid evolution of hermaphroditic mating systems. By contrast, genes that control germ cell fate in other animals have pleiotropic functions that prevent similar changes.

18:15 Sex pheromone in nematode: developmental synthesis, perception and evolutionary implication

King Chow
(bokchow@ust.hk)

Authors: So, W.K., C.M. Chan, M.Y. Wu, W.S. Yeung, and K.L. Chow
Address: Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

Abstract: Two different mating systems are adopted by *Caenorhabditis* species, dioecy and androdioecy. For dioecious species, finding a mating partner is an obligatory process for survival. We have reported the identification of a sex-specific attractant produced by females of the dioecious *Caenorhabditis remanei* for attracting mating partners. The nature and chemical constituents, the locale of its production, its cellular and molecular requirement for perception have been characterized. GC-MS analysis of developmental samples pin-pointed two candidate chemicals in the attractant, the exact identities of which will be confirmed by chemical synthesis coupled with bioassays. These attractant substances are produced by the somatic gonad as confirmed by laser ablation of gonadal progenitor cells in different combination. Their activity could be abolished by mating with males and resumed in the absence of further mating. While perception of these attractants could be observed in males of androdioecious *C. elegans*, the perception pathway was delineated by repeated testing of various genetic mutants affecting specific cell lineage or molecular function. We have defined the requirement of two different sensory neurons and an interneuron for this function. Moreover, a signaling pathway dependent of G-protein coupled receptor, subjected to further molecular modulation, is crucial for this pheromone perception process. Since most of the dioecious nematode species so far tested produce attractant chemical of sex pheromone nature, conservation of the pheromone perception pathway across different species and a hypothesis integrating the role of sex pheromone and the evolution of mating system will be discussed.

19:00 Dinner

THURSDAY, 25TH MAY

9:00 Consequences of mutation in the genome of *C. elegans* MA lines and natural isolates

Kelley Thomas
(kelley.thomas@unh.edu)

Authors: Thomas, W.K.[1], D.R. Denver[1,2], K. Morris[1]
Address: [1]Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824, USA
[2]Department of Zoology, Oregon State University, Corvallis, OR 97331 USA

Abstract: Mutation is an important force in biology, yet our understanding of the baseline rate and pattern and of mutation is limited. Using a set of artificially evolved lines of *C. elegans* that have accumulated mutations (MA lines) in the relative absence of natural selection, it has been possible to explore mutation in the relative absence of natural selection and compare these rates and patterns to that observed during natural line divergence. Now that we understand some aspects of mutation and selection in *C. elegans*, it is possible to ask about the consequences of those mutations on genome function, namely transcription and translation. By comparing transcription profiles of MA lines and natural isolates of *C. elegans* it has been possible to explore and characterize the role of selection and mutation on the divergence of gene expression in MA lines. These results suggest a strong role for stabilizing selection in natural populations. With any luck, we will extend this analysis one step further to ask questions about the next step in gene expression by performing parallel studies of the divergence of the proteome in MA and natural lines.

9:15 Mutation and Genome Evolution in *Caenorhabditis elegans*

Dee Denver
(denvedee@cgrb.oregonstate.edu)

Address: Department of Zoology, Center for Genome Research and Biocomputing Oregon State University, 2000 Cordley Hall, Corvallis, OR 97331 USA

Abstract: Mutation is the fuel for evolution in all forms of life and is ultimately responsible for all genetic variation. Despite its central role in biology, direct and unbiased estimates of genome-wide mutation processes remain elusive. An accurate understanding of spontaneous mutation processes requires a system where mutations can be characterized genome-wide, and the effects of natural selection are eliminated. A set of *C. elegans* mutation-accumulation (MA) lines, propagated across hundreds of generations as single randomly-selected hermaphrodites (N_e = 1), provide a system where mutations can be studied in the virtual absence of natural selection. Comparing mutation processes in the MA lines to molecular variation in *C. elegans* natural isolates provides an opportunity to evaluate the relative roles of mutation and natural selection in shaping *C. elegans* genome evolution. I will first discuss exciting new insights into molecular mutation and evolutionary phenomena that have resulted from studies involving the MA lines, then follow with findings from DNA-repair deficient MA lines that shed light on the intrinsic forces that shape mutational properties.

9:45 Evolution via compensatory mutations in *C. elegans*

Andy Peters
(adpeters1@wisc.edu)

Authors: Peters[1], A.D., B. Davis, and M. Whitlock[2]
Address: [1]Department of Zoology, Birge Hall, 430 Lincoln Dr.
University of Wisconsin-Madison, Madison WI 53706, USA
[2]Department of Ecology and Evolutionary Biology
University of British Columbia, Vancouver, Canada

Abstract: Deleterious mutations are expected to have potentially profound effects on the survival and evolution of populations, particularly small ones. However, these effects may be ameliorated if beneficial mutations are sufficiently common; particularly if mutations at other loci have the potential to compensate for deleterious mutations. We present an experiment to explore the scope for mutations at other loci to compensate for fitness loss due to knockout mutations. We show that the recovery of fitness is rapid in the face of a variety of fitness-reducing knockout mutations, and under surprisingly small population sizes.

10:15 Break

10:45 Genetic Variation and Experimental Evolution

Henrique Teotónio
(teotonio@igc.gulbenkian.pt)

Address: Instituto Gulbenkian de Ciência
Apartado 14, 2780 Oeiras, Portugal

Abstract: Despite its status as a model organism *C. elegans* has been poorly characterized in terms of natural genetic variation. Recently, a series of studies have begun this characterization and have found that despite its breeding system *C. elegans* harbors significant quantitative genetic variation for life-history characters, especially those related to outcrossing. While the use of selection experiments in the laboratory has helped our understanding of the genetic basis of some of these characters, little has been done on the study of adaptive processes. This is possibly the next frontier in *Carnorhabditis* evolutionary genetics studies, given that some problems are difficult to tackle with other model organisms, such as the the evolution of breeding systems. I will briefly review some of the studies on the quantitative genetics of life-history phenotypes and adaptation.

11:00 Inbreeding and outbreeding depression in *Caenorhabditis*

Elie Dolgin
(elie.dolgin@ed.ac.uk)

Authors: Dolgin, E.S., B. Charlesworth, and A.D. Cutter
Address: Institute of Evolutionary Biology, University of Edinburgh,
Ashworth Laboratories, King's Buildings, West Mains Road
Edinburgh EH9 3JT, UK

Abstract: The nematode *Caenorhabditis elegans* is composed primarily of selfing hermaphrodites, yet natural populations likely contain rare males. Males may be maintained in the species as a byproduct of selection against the accumulation of deleterious mutations by promoting genetic outcrossing and reducing any effects of inbreeding depression. To investigate this issue, we measured life-history traits in selfed versus outcrossed *C. elegans* that derived from recently isolated natural populations. The hermaphrodite-male breeding system (androdioecy) of *C. elegans* is thought to have evolved from a dioecious, male-female ancestor. To inform on how mating systems influence levels of inbreeding depression, we performed a parallel assay using similar methodologies with wild populations of the related dioecious *C. remanei*. We also maintained inbred lines of *C. remanei* through 13 generations of full-sibling mating. Inbred *C. remanei* showed dramatic reductions in productivity-related traits compared to outcrossed individuals, and this decline in fitness accumulated with increased inbreeding with little evidence of purging. In contrast, inbred strains of *C. elegans* performed better than crosses between strains, indicative of outbreeding depression. The causes and consequences of our results are discussed with respect to the evolution of androdioecy.

11:30 Understanding Genetic Variation in Rhabditid Nematodes

Matthew Salomon
(msalomon@zoo.ufl.edu)

Authors: Salomon, M.P., L. Levy, D. Blauton, W. Bour, T. Keller, D.G. Ostrow,
N. Phillips, J.Rosenbloom, C.F. Baer
Address: Department of Zoology, University of Florida
Gainesville, FL 32611-8525, USA

Abstract: The question "What maintains genetic variation?" dates to the earliest days of population genetics and remains contentious 75 years on. The question stems from two seemingly contradictory facts: the vast majority of observable mutations are also observably deleterious, and yet heritable genetic variation is plentiful. Two extreme alternatives are Mutation-Selection Balance (MSB), which posits that most standing genetic variation results from the recurrent input of deleterious mutations that are subsequently removed by purifying selection, and Balancing Selection (BS), in which natural selection acts to maintain genetic variation. The alternatives are not mutually exclusive; for example, it is possible that variation is maintained by MSB for some traits and by BS for others. The simplest explanation is that the trait in question is not under selection at all, in which case the standing variation represents a balance between neutral mutation and genetic drift.
Population genetic theory predicts some straightforward relationships between the standing genetic variance, V_G, and the genetic variance introduced by new mutation, V_M. Under certain circumstances, these relationships between V_G and V_M can shed light on the forces underlying phenotypic evolution. For example, at mutation-drift equilibrium the standing genetic variance for a neutral trait $V_G \propto 2N_eV_M$, where N_e is the genetic effective population size. If N_e is known, the ratio V_G/V_M can be used to infer the nature and approximate magnitude of selection acting on the trait in question. $V_G/V_M < 2N_e$, implies the trait in question is undergoing purifying selection; $V_G/V_M > 2N_e$, implies that balancing selection maintains genetic variation. Similarly, if the time since divergence (t) of two taxa is known, $V_G/V_M < 2t$ implies selection is purifying and $V_G/V_M > 2t$ implies diversifying selection has caused populations to diverge faster than the neutral rate. Alternatively, V_G/V_M can be interpreted in terms of expected persistence time (in generations) of a new mutation; the more deleterious the allele, the more quickly it will be removed by selection and the smaller the ratio V_G/V_M.

Over the past several years our lab has been investigating the properties of new mutations in a model nematode system. Mutations have been allowed to accumulate in the (relative) absence of natural selection, thus allowing us to estimate V_M for two species of Rhabditid nematodes, *Caenorhabditis elegans* and *C. briggsae*. However this begs the question, what is the relationship of V_M to V_G and what can this tell us about the nature and magnitude of selection acting on these species? Thus to complement our previous studies and generate an estimate of V_G within *C. elegans* and *C. briggsae*, estimates of V_G in "wild" worms are needed. Beginning in the summer of 2005 we assayed approximately 40 wild strains of *C. elegans* and 8 wild strains of *C. briggsae* for fecundity and body size at 20°C. These strains represent a worldwide collection that

was obtained through the Caenorhabditis Genetics Center at the University of Minnesota. The primary goal of this study is to characterize the extent of standing life-history variation in two species of Rhabditid nematodes; therefore data from a representative worldwide collection is needed. Comparisons of V_M to V_G between our mutation accumulation lines and the world wide isolates will allow us to draw strong inferences about the magnitude and pattern of constraint on phenotypic evolution, as well as provide valuable information about the forces responsible for the genetic diversity within and between species.

12:00 Lunch

13:00 Poster Session and Informal Meetings

17:30 Genetic Analysis of Complex Phenotypes

Jan Kammenga
(Jan.Kammenga@wur.nl)

Authors: Alvarez, O.A., E.W. Gutteling, J.Fu, Yang Li, J. A.G. Riksen, P. Prins, R.C. Jansen, and J.E. Kammenga
Address: Laboratory of Nematology, Wageningen University 6709 PD Wageningen, The Netherlands

Abstract: Over the last decades it has become widely acknowledged that environmental change, formerly regarded as a nuisance factor troubling the interpretation of genetic observations, is one of the most important drivers of evolution by natural selection. Gene-environment interactions are at the heart of this selection process affecting traits in numerous organisms. Significant progress has been made in the understanding of gene-environment interactions in various species. These, and all other such studies involved expression analysis of single genes or whole genome expression profiles aiming to identify up- and down regulation at different environmental conditions. However it is unknown to what extent environmental cues affect heritable variation in gene expression and how the environmental effects influence gene pathways underlying complex and polygenic traits. Previously we found strong genetic variation for *C. elegans* traits in response to temperature in a recombinant N2 x CB4856 inbred panel. We than studied the heritable variation of gene expression by analyzing 22,490 transcripts covering the full genome of *C. elegans* at 16°C and 24°C using a distant pair wise approach and subsequently hybridizing the extracted RNA for each temperature using 40 oligonucleotide micro-arrays. Subsequent QTL mapping revealed that a change in environmental temperature from 16°C to 24°C resulted in 22% new expression linkages in *C. elegans*, most of which were trans-acting. Three times as many linkages were found at 24°C than at 16°C. Transcripts involved in neuronal pathways showed increased linkage at 24°C. The genes detected show that at 24°C there is increased activity in neuronal pathways underlying environmental detection coupled to locomotion and developmental processes such as growth and reproduction. At 16°C neurosensing of the environment seems to be of primary importance. These results indicate that environmentally induced differential linkage of gene expression is widespread across the genome and that nearly one quarter of all transcript linkages are affected.

17:45 Finding the nucleotides underlying phenotypic variation in *C. elegans*

Matthew Rockman
(mrockman@princeton.edu)

Authors: Rockman, M., and L. Kruglyak
Address: Lewis-Sigler Institute for Integrative Genomics Princeton University, Princeton, NJ 08544, USA

Abstract: Phenotypic differences among species originate as heritable variation within populations. A central goal for evolutionary biology must be to describe the genetic basis of such variation and to discover the evolutionary forces that generate and maintain it. We are mapping the variants underlying phenotypic variation in C. elegans using a panel of 240 highly recombinant inbred lines derived from a 10-generation advanced intercross of the N2 and CB4856 strains. We have genotyped these RILs at 1400 informative SNP loci evenly distributed across the C. elegans chromosomes. We have also genotyped 125 wild strains of *C. elegans* in order to estimate the recombinational history of the genome and to characterize patterns of linkage disequilibrium. The linkage and LD data allow us to pinpoint nucleotide variants underlying phenotypic

variation and to make inferences about their evolutionary histories. We will report mapping results and experimental confirmation for morphological and behavioral phenotypes.

18:15 An evolutionary perspective in innate immunity and pathogen defense

Hinrich Schulenburg
(hinrich.schulenburg@uni-tuebingen.de)

Address: Department of Animal Evolutionary Ecology
University of Tuebingen
72076 Tuebingen, Germany

Abstract: Infection by a pathogen represents one of the major threats to any living organism. Therefore, the availability of an efficient immune system, which permits recognition and subsequent elimination of a pathogen, is of high adaptive value. Moreover, alternative defenses are likely to evolve, including physical barriers and avoidance behaviors. Since the expression of any type of defense is likely to be energetically expensive, a trade-off of the available resources among different fitness-related traits is likely to ensue. To date, the dynamics of immune system evolution, the importance of alternative defense strategies, and the involvement of a life-history trade-off are as yet only poorly understood. Our group attempts to exploit the advantages of *C. elegans* as a tractable model system for an evolutionary analysis of innate immunity and pathogen defense. During this presentation, I will highlight our recent results on this topic. Based on a summary of our current understanding of *C. elegans* defenses, I will elaborate the importance of "evolution by gene duplication" in potential pathogen recognition receptors (e.g. the C-type lectins) and immune effectors (e.g. the lysozymes). Our recent results strongly suggest that evolutionary differentiation indeed led to functional diversification, thus increasing the competence of the immune response. Furthermore, I will discuss the importance of insulin-like signaling as a major life-history switch, which appears to mediate investment in either diversity of defenses (including both physiological and behavioral responses) or high metabolic rates, reproduction, and development. Using C. elegans to address these different facets of host–pathogen interactions provides a fresh perspective on our understanding of the structure and complexity of innate immune systems and the defensive repertoire against pathogens.

19:00 Dinner

FRIDAY, 26TH MAY

9:00 Evolution of Development in *C. elegans*

Ricardo Azevedo
(razevedo@uh.edu)

Address: Dept. Biology and Biochemistry,
University of Houston, Houston, TX 77204-5001, USA

Abstract: Genes typically influence complex multicellular phenotypes indirectly, through developmental processes. Thus, the study of development is important for understanding phenotypic evolution. From an evolutionary perspective, evolution of development (affectionately called "evo-devo") consists of two complementary research programs. Pattern evo-devo concentrates on the idea that, to understand phenotypic change we must know what the developmental mechanisms underlying the trait are, and how these mechanisms have evolved. Process evo-devo tries to elucidate how evolutionary forces, such as mutation, environmental change and natural selection, operate on developmental mechanisms, and, conversely, how development interacts with these forces to direct, bias and constrain phenotypic evolution. Here, I outline some of the major questions in process evo-devo and review some exciting contributions to the field using *C. elegans*.

9:15 Natural variation in the plasticity of the development of dauer larvae: quantitative trait mapping and fitness consequences

Simon Harvey
(Simon.Harvey@bristol.ac.uk)

Authors: Harvey, S.C., A. Shorto, and M.E. Viney
Address: School of Biological Sciences, University of Bristol
Bristol BS8 1UG, UK

Abstract: The choice between dauer and non-dauer development is one of the most crucial in the *Caenorhabditis elegans* life cycle. Development of young larvae into environmentally resistant dauer larvae is triggered by high levels of dauer pheromone, limited food availability and high temperatures. This switch between dauer and non-dauer development is therefore an example of phenotypic plasticity, where environmental conditions determine the developmental fate of worms.

Genetically distinct natural isolates of *C. elegans* vary in their developmental response to dauer-inducing environmental conditions. In order to understand the genetics of this variation in plasticity and the fitness consequences we have created a set of recombinant-inbred lines (RILs) from crosses between N2, which has a high plasticity of dauer development, and a wild isolate (DR1350), which has a low plasticity.

A quantitative trait loci (QTL) mapping approach has been used to map genetic factors controlling the differences in plasticity between these lines. This has identified several regions containing candidate QTLs affecting the plasticity of dauer development. Nearly isogenic lines (NILs) have been constructed for one of these candidate QTLs. Analysis of these NILs has confirmed that the region contains genes that affect the plasticity of dauer larvae development. The fitness consequences of variation in this plasticity have been investigated by identifying fitness-related traits that are correlated with the plasticity of dauer larvae development in the RILs. This has identified a positive correlation between the population growth rate and plasticity. Further investigation of this has revealed that the variation in population growth rate is a consequence of variation between the RILs in the extent to which their reproductive success is affected by limited amounts of food.

These data have therefore developed a clearer picture of the genetics behind variation in a complex trait and, additionally, suggest the selective forces that may act to produce and maintain this variation.

9:45 Robustness and evolution of *Caenorhabditis* vulval development

Christian Braendle
(braendle@ijm.jussieu.fr)

Authors: Braendle C., J. Milloz, and M.-A. Félix
Address: Institut Jacques Monod, Tour 43, 2 place Jussieu,
75251 Paris cedex 05, France

Abstract: Organisms maintain developmental precision for most phenotypes despite changes in their internal and external environments. To better understand the mechanisms providing such robustness, we study the development of the vulva in *Caenorhabditis* species. This organ is formed by three precursor cells aligned in the ventral epidermis: P6.p adopts a 1° fate, P5.p and P7.p a 2° fate. In addition, P4.p, P8.p (and P3.p when it does not fuse to the epidermal syncytium) are competent to form vulval tissue but normally adopt a non-vulval 3° fate. Specification of the three fates involves a well-characterized network of intercellular signaling pathways, including EGF/Ras, Notch and Wnt. The partially redundant nature and complex regulation of these pathways seemingly ensure precision of this process. However, it is unclear how the interplay of these mechanisms may achieve precision.

We first measured the precision of vulval fate patterning and characterized deviant patterns for the *C. elegans* N2 genotype in six laboratory environments (standard laboratory conditions at 15°C, 20°C and 25°C; liquid; dauer; starvation). Overall, development appeared robust, with errors leading to a defective vulva occurring rarely in any environment (< 0.1%). In addition, different environmental conditions resulted in specific variants that did not result in a defective vulva. For example, starved N2 individuals were prone to miscenter their vulva on P5.p instead of P6.p – a variation exhibiting a wild type vulva due to P4.p competence. Additional isolates of *C. elegans* and *C. briggsae* also rarely exhibited vulval defects, yet differed in other variant patterns. After starvation, the *C. elegans* genotype JU258 showed a drastic increase in P4.p and P8.p fusion, but rarely miscentered the vulva whereas *C. briggsae* AF16 was prone to miscenter its vulva on the posterior cell, P7.p. In addition, P3.p fusion in AF16 significantly decreased after starvation while it increased in N2 and JU258. Thus, while vulval development is generally robust, it undergoes changes in response to the environment and these changes depend on the genotype.

We further characterized the phenotypic effect of random mutation on vulval development using mutation accumulation lines derived from N2 (a generous gift of L. Vassilieva, C. Baer and M. Lynch). Errors in vulval patterning and centering increased in most lines.

To understand how the use of the genetic network was affected by environmental change, we examined vulval development mutants in the same six environments. Different environments greatly affected the phenotypic effect of mutations. For example, similar to previous studies, we found that starvation and dauer environments strongly suppressed mutations reducing Ras pathway activity, whereas starvation enhanced a mutation reducing Wnt pathway activity. These results confirm that vulval development is responsive to environmental inputs that can modulate pathway use. We are now testing how the environment alters specific interactions between pathways and how this contributes to precision of vulval development. We are further analysing how certain environmental cues are perceived and mediated to affect vulval cell fate patterning.

Finally, we examined how such a robust developmental process evolves. For *C. elegans*, we introgressed several mutations into six divergent genotypes (CB4856, JU258, PS2025, AB1, PB303, PB306) and found that the phenotypic effect of a mutation varied greatly between genotypes (see contribution by J. Milloz). In different *Caenorhabditis* species, we ablated the anchor cell (the source of the EGF signal) during the induction process and found that the resulting fate patterns varied greatly (see contribution by M.-A. Félix). Thus we could reveal cryptic genetic variation in vulval formation. We are currently characterizing this evolutionary variation and test how it translates into variation in precision of vulval development.

10:15 Break

10:45 Evolution of Development in the *Caenorhabditis*:
Rapid Evolutionary Divergence of Polycomb-Like Proteins in *Caenorhabditis*

Scott Emmons
(emmons@aecom.yu.edu)

Address: Department of Molecular Genetics
Albert Einstein College of Medicine, Bronx, NY 10461, 718-430-3130, USA

Abstract: Histones are among the most conserved proteins and provide a common structure to chromatin in all eukaryotes. Histones play an essential role in governing accessibility of promoters to RNA polymerase and not surprisingly the multitude of proteins and protein complexes that regulate histone/chromatin architecture are also conserved across animals and plants, including nematodes. Thus it is unexpected to find that one major family of global regulatory proteins, the Polycomb Group (PcG) proteins belonging to the PRC1 complex, are not present in *C. elegans*. None of the four proteins in this complex, (in *Drosophila* they are *Polycomb, Polyhomeotic, Posterior Sex Combs, and dRING*) can be readily found by BLAST search in the genomes of *C. elegans*, *C. briggsae*, or *C. remanei*.

In *C. elegans*, the novel gene *sop-2* appears to serve the regulatory role of a PRC1 PcG protein. Mutations in *sop-2* result in ectopic Hox gene expression, as do PcG mutations in other animals, and affect expression of many other genes as well, suggesting a global regulatory role. Null mutations of *sop-2* are embryonic or early larval lethal. *sor-1*, a new gene recently identified by H. Zhang and coworkers, has similar genetic properties and SOR-1 physically interacts with SOP-2, suggesting that it functions in a complex with SOP-2. SOP-2 contains the protein-protein interaction SAM domain found in many proteins including Polyhomeotic. Presence of RNA binding domains, sumoylation, and localization to nuclear bodies are additional common characteristics strengthening the connection to PcG proteins. Remarkably, neither *sop-2* nor *sor-1* can be identified in the *C. briggsae* or *C. remanei* genomes. Phylogenetic analysis using the SAM sequence shows that the SOP-2 SAM domain defines a subfamily of five unrelated genes in the *C. elegans* genome sharing a similar SAM sequence. None of these five genes appears to be present in *C. briggsae*. The functions of the other four genes in this family are unknown.

These observations prominently raise the question as to the nature of the genes not conserved between *Caenorhabditis* species. Stein *et al.*, estimated that some 5% to 10% of the genes predicted in the *C. elegans* and *C. briggsae* genomes were species-specific or “orphan” genes, but these may be underestimates, since conservation in *C. briggsae* was used as a criterion in their gene-prediction algorithm. (*sop-2*, for example, was not among the original gene predictions in the *C. elegans* genome sequence.) Most likely, non-conserved genes were present in a common ancestor but have diverged so rapidly that their orthologs are no longer recognizable. However, an origin in horizontal transfer is also a possibility. In either case, an essential role in a fundamental process like global regulation of gene expression via chromatin architecture is unexpected. Identification and comparison of the genes in *C. briggsae* and *C. remanei* that play the corresponding regulatory function as *sop-2* and *sor-1* in *C. elegans* may help lead to an understanding of the mechanism by which these function to regulate gene expression and reveal how they

can rapidly vary independently of other chromatin components. Why they would be under apparent selection to do so remains a mystery.

11:00 Evolution of excretory system features in *Caenorhabditis*

Helen Chamberlin
(chamberlin.27@osu.edu)

Authors: Chamberlin, H.M., X. Wang, H. Jia
Address: Department of Molecular Genetics
Ohio State University, Columbus, OH 43210, USA

Abstract: Our group uses comparative genetics within the Caenorhabditis genus to understand the molecular changes responsible for phenotypic differences between species. Our work has focused on the excretory system which is responsible for maintaining osmotic homeostasis and eliminating waste. The excretory system in *C. elegans* is distinct from that in other Caenorhabditis species including *C. briggsae*, and *C. elegans* exhibit altered excretory duct anatomy and increased salt tolerance compared to the other species tested. These functional differences result from altered expression of a single gene in the *C. elegans* excretory duct cell, the *Ovo*-related zinc finger transcription factor gene *lin-48*. *C. elegans* expresses *lin-48* in the excretory duct cell, and other species such as *C. briggsae* do not. In addition, *C. elegans lin-48* mutants exhibit altered excretory system features similar to those found in the other species. Although changes in the regulation of this one gene are critical for the species differences, we find that multiple regulatory changes contribute to the expression changes. In comparing *C. elegans* to *C. briggsae*, we find there are difference in both *lin-48* cis acting sequences and trans acting factors. Our current work is to identify all the molecular changes responsible for the trait differences, to better understand how new gene regulatory features can evolve.

An analysis of the *C. elegans lin-48* (*Cel-lin-48*) regulatory sequences has identified several enhancer sequences that act to promote expression of the gene in the excretory duct cell. These enhancers act at different developmental times, and collectively are responsible for the stable expression of *Cel-lin-48* in the excretory duct cell from late embryogenesis through adulthood. The bZip transcription factors ATF-2 and CES-2 are essential for expression of *lin-48* in the excretory duct cell in the embryo, and influence the anatomical features of the duct. In contrast, the homeodomain transcription factor CEH-43 affects expression of *lin-48* in larvae and adults, and influences salt tolerance. These experiments show that multiple changes in the *lin-48* regulatory sequences are responsible for its altered expression pattern.

To identify the trans acting factors that have likewise undergone changes subsequent to the divergence of *C. elegans* and *C. briggsae*, we are taking both molecular and genetic approaches. We are testing the function of the *C. briggsae* gene corresponding to each of the factors we identify that regulate *lin-48* excretory cell expression. Genes for ATF-2, CES-2, and CEH-43 are conserved in *C. briggsae*, and we are testing each for its ability to functionally rescue the corresponding *C. elegans* mutant. We are using two genetic screens to identify new genes that affect excretory system structure and function. One screen in *C. elegans* is for new mutants with altered (ancestral or *C. briggsae*-like) excretory system anatomy and reduced salt tolerance. This screen has identified one mutation in a new gene, *gu32*. We are currently mapping the mutation and characterizing the mutant phenotype. A second screen in *C. briggsae* is a selection for mutants with increased salt tolerance. A screen of 500,000 EMS mutagenized gametes has identified ten mutations that confer increased salt tolerance. Of these ten, two also confer altered (*C. elegans*-like) excretory system anatomy. We are currently testing whether these mutations alter *Cbr-lin-48* function, and mapping the mutations to the *C. briggsae* genetic map. We are hopeful that complementary genetic strategies in the two species will identify new genes important for excretory system structure and function, as well as determine the range of molecular changes that contribute to the species differences.

11:30 Evolution of vulval development in *Caenorhabditis* nematodes

Bhagwati Gupta
(guptab@mcmaster.ca)

Authors: Marri, S., B. Nagagireesh, and B.P. Gupta
Address: McMaster University,
Hamilton, ON L8S 4K1, Canada

Abstract: We are interested in understanding the evolution of developmental mechanism during animal development. Towards this, we are doing comparative analysis of the vulval patterning in the nematode *C. briggsae* that is a close relative of *C. elegans*. The ease of genetic manipulation in *C. briggsae* is comparable

to that of *C. elegans*. The two nematodes have nearly identical morphology although recent studies have shown subtle differences in the underlying mechanisms.

We are taking various experimental approaches towards identifying the vulval genes in *C. briggsae* and studying their biological function. We have carried out large scale genetic screens to isolate *C. briggsae* mutants that exhibit vulvaless (Vul), multivulva (Muv) and protruding vulva (Pvul) phenotypes. The genetic analysis of some of these mutants has revealed differences in the pattern of cell proliferation and differentiation compared to the *C. elegans*. Current experiments focus on the mapping and molecular analysis of the involved loci. The findings will help understand whether vulval development in *C. briggsae* is mediated by the altered regulation of existing *C. elegans* genes or by new genes that have acquired vulval function. These experiments will generate necessary tools to study other interacting genes and pathways that may have evolved to confer distinct cell fates in the developing vulva.

12:00 Lunch

13:00 Poster Session and Informal Meetings

15:45 Genomics and Resources for the *Caenorhabditis* scientific community

Marie-Anne Félix
(felix@ijm.jussieu.fr)

Address: Institut Jacques Monod, Tour 43, 2 pl. Jussieu,
75251 Paris Cedex 05, France

Abstract: Genome sequencing of several *Caenorhabditis* species has been driving genome evolution studies, and provides a great tool for experimental studies using these species. More generally, an aim of this session is to provide information on available resources of various kinds for the *Caenorhabditis* evolution community, and to discuss which further developments we may wish. A non-exhaustive list of such resources includes: collections of natural isolates; the genome sequences of different wild isolates and more *Caenorhabditis* species; genotyped recombinant inbred and near isogenic lines, mutation accumulation lines, etc.; DNA and RNA hybridization arrays; genetic maps and tools in other *Caenorhabditis* species; the integration of evolutionary relevant biological information into existing databases such as Wormbase.

16:00 A program that predicts nematode genes by combining well conserved exons predicts by existing gene finders

Avril Coghlan
(alc@sanger.ac.uk)

Authors: R. Durbin, and A. Coghlan
Address: Wellcome Trust Sanger Institute, Cambridge, England.

Abstract: Correct gene predictions will be crucial for most evolutionary analyses of the genomes of closely related nematode species. We present a program that predicts nematode genes by combining well conserved exons predicted by existing gene-finders. Our approach uses the degree of conservation between related nematode genomes to select the most plausible exons that were predicted by many different gene-finders.

We describe how our program was used to predict genes in the newly sequenced *Caenorhabditis remanei* genome, by using conservation to *C. elegans* and *C. briggsae* to score each of the alternative *C. remanei* exons predicted by multiple gene-finders.

Initial results using a test set of confirmed C. elegans genes are promising: about 72% of the exons predicted by our algorithm are correct. This approach is likely to be very useful for predicting genes in the nematode genomes that are currently being sequenced, such as *Caenorhabditis sp*. PB2801 and *C. japonica*.

16:30 Cis-regulatory analysis of three *Caenorhabditis* genomes

Erich Schwartz
(emsch@its.caltech.edu)

Authors: Schwarz, E.M., A. Mortazavi, S.G. Kuntz, A.J. Saldanha, J.A. DeModena, D. Fu, T. De Buysscher, H. Shizuya, J.C. Roden, B.J. Wold, P.W. Sternberg
Address: Division of Biology, 156-29, California Institute of Technology, Pasadena, CA, 91125, U.S.A.

Abstract: We scanned three *Caenorhabditis* genomes (*C. elegans*, *C. briggsae*, and the newly sequenced *C. remanei*) for conserved non-coding DNA elements which could indicate cis-regulatory signals. We first used MUSSA, an *n*-species generalization of Family Relations, to select ungapped blocks of high identity. MUSSA identified ~2% of the non-coding DNA in the *ceh-13*/*lin-39* Hox cluster as conserved between *C. elegans*, *C. briggsae*, and *C. sp.* CB5161. Within this restricted subset, transgenic assays of Hox cluster elements show expression patterns for 5 of 7 tested. Fine-scale examination of other experimentally verified cis-regulatory elements in *lin-3* and *lin-11* shows that they are closely associated with MUSSA-identified elements, but not identical to them. We hypothesized that this loose association of small regulatory motifs with large conserved blocks will be generally valid. We thus carried out a genome-wide analysis with the Cistematic open-source framework. Cistematic used MUSSA to find blocks of non-coding DNA in the flanks and introns of 8,100 genes with orthologs in all three genomes. These blocks were then scanned for up to 5 candidate position-weight matrices (PWMs) per gene with MEME, and the PWMs were purged of degenerate and redundant instances. The resulting 25,300 PWMs were used to re-scan all three genomes, checking for conservation and significantly associated GO terms; this biased the motif finders towards conserved motifs in MUSSA blocks, but allowed genome-wide analyses to be unrestricted by MUSSA. We found 2,800 conserved motifs associated with defined biological functions (as indicated by GO terms), which we are now analyzing for coexpression by microarray and anatomical data in WormBase. We also tested *in vivo* Cistematic's ability to find regulatory elements in coexpressed collagen genes; deletion of Cistematic-predicted elements from collagen promoter regions reliably altered their regulation of reporter genes.

17:00 *C. briggsae* genetics team presentation: Integrating genetic and genomic resources for *C. briggsae*
Daniel Koboldt, Bhagwati Gupta, Scott Baird, Eric Haag, Helen Chamberlin and Ray Miller

Abstract: *Caenorhabditis briggsae* is a key emerging model organism. Comparative genomic and genetic studies of *C. briggsae* with its related model organism *C. elegans* promise powerful new tools for the identification of eukaryotic gene products and pathways and the study of the conservation/divergence of these. *C. elegans* and *C. briggsae* are phenotypically nearly identical but they exhibit significant differences at the genomic sequence level. *C. briggsae* investigators agree that a key required experimental tool for the organism is a good genetic map tied to the draft sequence. The map is needed to facilitate forward genetics including mapping mutants useful to investigators in the field as a prelude to positional cloning of the mutants. We are constructing a dense genetic map of *C. briggsae* by genotyping single nucleotide polymorphisms (SNPs) on two sets of recombinant inbred strains. The map will anchor and orient contigs from the draft sequence, making the sequence more useful. We are also assembling collections of mutant animals and are genetically mapping the location of the mutations with high throughput methods using bulked segregants. We hope to use similar techniques to map many mutants found by investigators in the field, and we will make the data and mutants publicly available. The map tools provided by this work will enable a powerful new style of science for comparative genetics and genomics.

17:30 Group Discussion on common resources to the community and future perspectives.

POSTER ABSTRACTS

Hybrid incompatibility between *C. elegans* strains as a model for speciation

Michael Ailion
(michael.ailion@gmail.com)

Authors: M. Ailion, and E.M. Jorgensen
Address: University of Utah, Department of Biology
257 S 1400 E, Salt Lake City, UT 84112 USA

Abstract: I am interested in the mechanisms that underlie speciation and am using different natural isolates of *C. elegans* to study this process. One common model of speciation is that of hybrid incompatibility. Specifically, certain sets of genes are postulated to coevolve together. If populations are separated, the genes will diverge from population to population and though they function perfectly in each individual population, they may be incompatible with the genes that have evolved in a separate population, leading to hybrid incompatibilities between such populations. Such incompatibilities could further prevent the mixing of the genes of the two populations, thus driving speciation. However, in very few cases is it known what genes may be responsible for hybrid incompatibility.

I have begun studying this model using two diverged strains of C. elegans: the Bristol wild-type strain N2 and the Hawaiian wild-type strain CB4856. Both N2 and CB4856 have extremely low rates of embryonic and larval lethality (<1%). However, F1 hybrids between N2 and CB4856 exhibit a significantly higher rate of lethality (approximately 10%), indicating that there is some level of hybrid incompatibility between these strains. The lethality is usually in embryonic or L1 larval stages. Arrested L1 larvae have a characteristic lumpy phenotype, which could indicate defects in ventral enclosure of the embryos.

I am currently attempting to map the genetic loci responsible for hybrid incompatibility between N2 and CB4856 using SNP mapping technology. A large number of recombinant inbred lines between N2 and CB4856 have also been created in our lab and may prove useful for mapping.

Evolution and development of buccal cavity morphology in *Pristionchus*

Gilberto Bento
(gilberto.bento@tuebingen.mpg.de)

Authors: Bento, G.N., and R.J. Sommer
Address: Max-Planck-Institut für Entwicklungsbiologie, D-72076 Tübingen, Germany

Abstract: We study the role of developmental plasticity and modularity in evolution, and use nematodes of the genus *Pristionchus* to address these questions at an intra and inter-species level.

Pristionchus pacificus is a member of the Diplogastridae family, which was described as a novel species in 1996. Since then a genetic, molecular and genomic toolkit was generated, establishing *P. pacificus* as a major satellite model organism for evolutionary developmental biology. The systematic description of species of the *Pristionchus* genus with the construction of a robust molecular phylogeny, allows comparisons between closely related species.

Pristionchus species, like other members of the Diplogastridae family show a morphological dimorphism in the buccal cavity between eury- and stenostomatous worms. The variation in stomatal morphology in Diplogastridae is thought to be related to different feeding habits.

The first objective of this project is to understand the genetic basis of these alternative phenotypes: is it a genetic polymorphism or phenotypic plasticity underlying the presence of the two morphologies?

Using *P. pacificus* strain California, 20 inbred selection lines were initiated for each mouth form. Both eury- and stenostomatous lines had a ratio of approximately 30% eurystomatous and 70% stenostomatous after 9 generations, indicating that this is a case of developmental plasticity, and that in the California strain there is no genetic variation for plasticity.

The same experimental procedure was than used for 10 inbred selection lines per mouth form, for 4 different *P. pacificus* strains and 1 strain of *Pristionchus entomophagous*, to understand if there is genetic variation for the plasticity within *P. pacificus* and between different *Pristionchus* species. The results indicate that there is genetic variation for plasticity between the *P. pacificus* strains that we used, and between *P. pacificus* and *P. entomophagous*. The analysis of recombinant lines, from crosses between two of the previous used strains suggests that this is a one-locus trait.

We have started to investigate the environmental control of this trait. The addition of Acridine orange to OP50 (used as food), caused a general trend towards a greater proportion of stenostomatous morphs in all strains. We are now

analysing the result of changing pH of the environment. We have also changed the food source to *Mixococcus* which caused unconsistent changes in the ratios of the mouth morphologies.
All together, our results so far show that this is a case of a polyphenism, subject to environmental control, and that there is genetic variation in natural populations for the reaction norm of this trait. The changes in the reaction norm are apparently due to variation in one single locus.

The same experimental procedure is being used for 10 inbred lines per mouth form, for 4 different *P. pacificus* strains and 1 strain of *Pristionchus entomophagous*, to understand if there is genetic variation for the plasticity within *P. pacificus* and between different *Pristionchus* species.

The second question to be asked is: which environmental factors influence the plastic response? In the California strain of *P. pacificus*, different inbred lines are exposed different feeding environments. We are testing the following food sources: OP50 strain of *E.coli*; other strains of *E. coli*; the fungi *Beauvaria bassiana*; *Saccharomyces cerevisae*; and other nematode species. We will also test for the influence over the plastic response of overcrowding conditions and passage through dauer stage.

Evolution of early embryogenesis in nematodes: a paradigm for comparative functional genomics.

Michael Brauchle
(mab404@nyu.edu)

Authors: Brauchle, M., and F. Piano
Address: Department of Biology, New York University,
NY 10003-6688, USA

Abstract: A fundamental question in developmental biology is how different species use a similar set of genes to generate developmental diversity. To approach this question we have been probing how developmental networks evolve during early embryogenesis in Rhabditidae. Using time-lapse microscopy we analyzed 32 species from the 1 to the 4-cell stage and defined 45 binary characters that describe each species. We used these data to map the evolution of early embryonic events on an established molecular phylogeny, and have built a web-accessible database to navigate these data. In addition to comparing embryogenesis across species, we also compared these data with previous genome-wide RNAi studies in C. elegans. We hypothesize that *C. elegans* RNAi phenotypes that resemble the wild type phenotypes of other species help connect which molecular networks are giving rise to the phenotypic changes.

To test this idea we have been focusing on Protorhabditis and Diploscapter spp., in which early cleavages follow a pattern resembling that produced in C. elegans only after mutation or RNAi of particular par genes. The par genes define a group of genetically and physically interacting proteins that are required to establish polarity. Correct par function in C. elegans leads to asymmetric cell divisions that establish different cell lineages and consequently pattern the developing embryo. Inferring from the *C. elegans* par network we predicted that the par-1 kinase would be localized differently in Protorhabditis or Diploscapter spp. as compared to *C. elegans*. To test this hypothesis we raised antibodies against a conserved domain of PAR-1 and found a different localization of PAR-1 in species from Protorhabditis or Diploscapter. Finally, to further probe how rapidly the function of the par genes may be evolving we are using RNAi across Caenorhabditis species to ask what role these genes play in these species. Together, these analyses should help setup a system to address basic questions in evolution of development and suggest an important role for the par network in generating evolution of early embryonic development.

Complex configuration of epidermal cells underlying cuticular feeding appendages in Acrobeles complexus (Nematoda: Rhabditida)

Daniel J. Bumbarger
(bumbad01@student.ucr.edu)

Authors: Bumbarger[1], D.J., J. Crum[2], M. Ellisman[2], J.G. Baldwin[1].
Address: [1] Nematology, University of California, Riverside, CA, USA;
[2] National Center for Microscopy and Imaging Research,
University of California, San Diego, CA, USA.

Abstract: Acrobeles complexus is a free-living microbial-feeding nematode with two rings of elaborate cuticular appendages (probolae) surrounding the buccal cavity. There is a gradient in morphology with some taxa having simple probolae and others, including A. complexus, with elaborate structures. Molecular phylogenies suggest that probolae evolved relatively rapidly making the group suitable for tractable questions about the evolution of novelty. Here, we have

reconstructed the shape and nuclei of 13 cells in the "nose" from serial sections in adults and juveniles. Nuclei in first stage juveniles are large and readily resolved with light microscopy. Also reconstructed are several relevant cytoskeletal features in the pair of ring-like arcade syncytia. The numbers of nuclei associated with cells in the nose is identical to *Caenorhabditis elegans*, and an additional cell was discovered in A. comlexus between the hyp-3 and hyp-4 homologs. Cell shape underlying probolae is unexpectedly complex, and provides the bases for hypotheses on how they are formed. The Hyp-2 *C. elegans* homologwas found to have projections underlying the cuticle of the inner ring of probolae. Surprisingly, the socket cells of the inner labial sensillae seem to be responsible for several aspects of cuticular morphology including the outer ring of probolae. Complex spatial relationships between the arcade cells, the hyp-2 homolog and probolae suggest that they may also play a role in the formation of probolae. Much of the information gained from reconstruction of A. complexus is not available for any other nematode, including *C. elegans*, making comparisons difficult. Work is currently underway to compare specific elements of cell shape in A. complexus to homologs in C. elegans; one goal of these comparisons is to better understand the evolution of complex morphologies i.e. feeding appendages in Rhabditida.

The cost of dioecy under different mutational loads in *C. elegans*

Sara Carvalho
(saracarvalho@igc.gulbenkian.pt)

Authors: Carvalho, S. and H. Teotónio
Address: Instituto Gulbenkian de Ciência, Apartado 14, 2780 Oeiras, Portugal

Abstract: The phenomenon of sexual reproduction and the evolution of breeding systems hold some of the most interesting paradigms in Evolutionary Biology. Explanations for the origin and maintenance of androdioecy in particular – a system where males and self-fertile hermaphrodites coexist – greatly rely on the magnitude of mutational effects and its generation of inbreeding depression, which have been scarcely supported by empirical evidence. We addressed the hypothesis of the evolution of androdioecy from dioecy in different mutational and genetic contexts. Wild-type hermaphrodites of two *Caenorhabditis elegans* strains were delivered to dioecious (fog-2 null mutant) strains. Additionally, donor and recipient strains with different levels of mutation loads were employed. These mixed populations were maintained under a constant laboratory environment for ten generations. We estimated the frequency of males as an indirect measure of the outcrossing rate, the frequency of fog-2 null mutants, viability and population competitive for different time points. Our results provide insights into the evolutionary forces that may underlie the maintenance sexual reproduction.

Experimental evolution of sex determination and reproductive isolation in *Caenorhabditis elegans*

Chris Chandler
(cholden@iastate.edu)

Authors: C.H. Chandler[1], P.C. Phillips[2], and F.J. Janzen[1]
Address: [1]Department of Ecology, Evolution, and Organismal Biology
Iowa State University, Ames, USA
[2]Center for Ecology and Evolution, University of Oregon, Eugene, USA

Abstract: Sex determination mechanisms in animals represent an evolutionary paradox. On one hand, the primary sex-determining signals vary widely among species, from diverse genotypic sex determination (GSD) systems to various forms of environmental sex determination (ESD), such as temperature-dependent sex determination (TSD). On the other, a remarkable degree of conservation is seen in the homology of the most downstream elements in sex determination pathways, even among taxa separated by hundreds of millions of years. It has been proposed that this pattern of upstream divergence and downstream conservation may have arisen via the recruitment of new upstream regulators to the pathways, due to selection to strengthen the sex-determining signal and to restore a balanced sex ratio. While this hypothesis has been supported by some comparative data, it has been difficult to verify experimentally. Subjecting populations of a rapidly developing model organism such as *Caenorhabditis elegans* to selection to alter their sex determination pathways, however, may provide a direct test of this hypothesis. Here, we describe thermal reaction norms for sex ratio in two strains of *C. elegans*, CB5362 and CB6415, which exhibit TSD patterns similar to those naturally occurring in many reptiles. By rearing these nematodes in environments that produce skewed sex ratios, we will promote selection to modify the sex determination pathway to restore a balanced sex ratio, either by altering the thermal reaction

norm for sex ratio or eliciting a switch to a GSD mechanism. Afterwards, we will sequence several sex determination genes and their regulatory regions to identify the genetic basis of any changes in sex determination and to search for new regulatory elements that may have led to the recruitment of new genes to the sex determination pathway. We will also explore using other techniques such as QTL mapping to identify loci that contribute to quantitative variation in the pivotal temperature of sex determination (the temperature at which a 1:1 sex ratio is produced) and thermal reaction norm for sex ratio. Finally, at the conclusion of the experiment, we will cross different replicate lines to explore how changes in sex determination may play a role in the early stages of reproductive isolation.

Robustness and evolution of a cellular process: the antero/posterior polarization of early Caenorhabditis embryos

Marie Delattre
(marie.delattre@isrec.ch)

Address: ISREC, 1066 Epalinges/Lausanne, Switzerland

Abstract: Robustness to genetic and environmental perturbations is thought to allow the "silent" evolution of underlying signaling networks. Under certain conditions, these cryptic modifications may be revealed and trigger evolutionary changes. We chose to explore the robustness and evolution of a cellular process: the antero/posterior polarization of one-cell stage Caenorhabditis embryos. First because embryonic polarity is a fundamental cellular process, relatively simple and well characterized in C. elegans. Second because this process is highly reproducible within species; arguing for its robustness. Third, the A/P polarity of the C. elegans zygote is depending on the sperm entry at fertilization while it is not the case for some distantly related species (such as Acrobeloides sp.). This shows that the process of early A/P polarization has changed during nematodes evolution and pushed us to search for evolutionary modifications within species.

Our working hypothesis is that within the C. elegans species and between closely related species (within the Caenorhabditis genus), the process of early A/P polarity is apparently conserved, even though the underlying molecular mechanisms have been modified. We propose to experimentally reveal these cryptic genetic variations.

First we will compare several strains of the C. elegans species. These strains will be subjected to extreme environnemental or genetic conditions, and the polarization of embryos will be followed under these circumstances. This should abolish the buffering of the system and reveal differences between the different genetic backgrounds. For instance, if one strain has polarity defects under high temperature conditions or after starvation while the others are not affected, this will show that this strain has a modified signaling network. On the other hand, we will introgress in different strains mutated genes affecting the polarization of C.elegans N2 embryos. For instance, if one allele has no effect in one strain, while it gives 50% polarity defect in N2, this will show that this specific strain has bypassed the requirement for this gene or acquire redondant pathways. Second we plan to compare species of the Caenorhabditis genus which are seemingly similar for the A/P polarization of their one-cell stage embryos (i.e the position of the sperm dictates the A/P polarisation of the zygote). These species have the same experimental advantages as C. elegans. For some of them, their genome is sequenced, RNAi and transgenesis have been established. Therefore, it is possible to undertake a detailed analysis of the molecular mecanisms at work for the A/P polarization of early embryos, and compare it to the C. elegans dataset. We will concentrate on the following species : *C. briggsae*, *C. remanei*, *C. japonica*, *C*. sp.3 PS1010 et *C*. sp.4 CB5161.

Genetic variation and population structure in wild isolates of *Caenorhabditis elegans* collected from California.

Alivia Dey
(Dey@Biology.Rutgers.Edu)

Authors: Dey, A., and J. Hey
Address: Rutgers University, Nelson Biological Labs (rm B326),
604 Allison Rd., Piscataway, NJ 08854-8082, USA.

Abstract: Though widespread in distribution, the nematode worm *Caenorhabditis elegans* exhibits low levels of genetic variation at the DNA sequence level, a paradox which may be partly explained its typical self-fertilization mode of reproduction. However recent work on the genetic structure of natural populations of *C. elegans* suggests a substantial level of outcrossing together with very strong local population structure.

In the present study, genetic variation is studied by detecting single nucleotide polymorphisms in a random genome-wide manner using Amplified Fragment Length Polymorphism analysis (AFLP). The worms used in this study are natural isolates of *C. elegans* collected from parks and gardens around the Los Angeles area in southern California. Some populations sampled were a few meters apart, enabling the assessment of outcrossing and population structure on a very local scale.

Adapting to variable environments: life-history strategies in *C. remanei* lineages

Anaid Diaz
(s.diaz-palacios.1@research.gla.ac.uk)

Authors: Diaz, A., J. Lindstrom, and D. Haydon
Address: Rm 303, Graham Kerr Bldg. Theoretical Ecology Group,
Division of Environmental & Evolutionary Biology,
University of Glasgow, Glasgow G12 8QQ, UK

Abstract: How does the evolutionary history of a population influence the extent to which it can adapt to future environmental change? This is an important question for natural populations adapting to global warming and other forms of environmental change, but controlled laboratory experiments with model species can inform the study of such evolutionary process. While Caenorhabditis spp. have been widely used to study genetics, neurobiology, embryonic development and ageing processes, the importance of environmental and ecological determinants on its demography and evolution are poorly understood. In this study we will use the gonochoristic species C. remanei to explore population adaptability to unpredictable environments. Three lineages started with sets of F1 hybrids of laboratory strains will be raised in three different environments: a constant temperature (23 deg C) environment, environments that predictably fluctuate at regular intervals between high (30 deg C) and low temperature regimes (16 deg C), and unpredictable environments fluctuating between these two regimes, subject to the constraint of equal time spent in each temperature regime. After 50 generations, lineages will be swapped between these environments. To simulate a deteriorating environment, lineages will also be placed in an environment in which temperature will be decreased monotonically. Population life-history parameters such as phenology (egg laying and hatching time), offspring productivity, population growth rate and sex ratio will be monitored every 10 generations from sub-samples taken throughout these experiments. Preliminary results of this experiment will be presented and discussed.

CB4856 /N2 Introgression Lines of *Caenorhabditis elegans* – a permanent population for QTL fine-mapping

Agnieszka Doroszuk
(Agnieszka.Doroszuk@wur.nl)

Authors: Doroszuk, A., E. Fradin, B. Waterbeemd, J. Riksen, and J. Kammenga
Address: Laboratory of Nematology, 6709 PD Wageningen, The Netherlands

Abstract: Segregant *C. elegans* populations of the canonical strain N2 and strain CB4856 are increasingly used for mapping quantitative traits such as feeding behaviour and neurological disorders. The power of QTL detection for these complex traits depends to a large extent on the type and size of the mapping population. To facilitate mapping strategies and to overcome typical shortcomings of many mapping populations like low resolution power, overshadowing effect of major QTL on minor QTL or interaction between unlinked QTLs, we have recently developed a permanent mapping population of introgression lines (IL) of the nematode *Caenorhabditis elegans*. For that purpose we crossed CB4856 with N2 and used the resulting progeny for selfing. Each of the introgression lines contains a single homozygous chromosome segment of CB4856 strain introgressed into the background of N2 as defined by a set of SNP markers. The mapping population consists of approximately 100 lines nearly isogenic to N2 with chromosomal segments of CB4856 covering in total 95% of the genome. We show properties of ILs as compared to recombinant inbred lines (RIL) population in QTL mapping of the response to extreme temperature stress (60ºC).

Characterization of *C. briggsae* germline sex determination mutants

Alana Doty
(adoty@umd.edu)

Authors: Alana V. Doty and Eric S. Haag
Address: Department of Biology, University of Maryland, College Park, MD, U.S.A.

Abstract: To investigate the evolution of germline sex determination in *Caenorhabditis*, we are conducting forward and reverse genetic screens in *C. briggsae* for Fog (feminization of germline) and Mog (masculinization of germline) mutants. Published and unpublished results from our lab and others indicate that both the "sperm on" and "sperm off–oocytes on" portions of germline sex determination are controlled differently in *C. briggsae* and the androdioecious model species *C. elegans*, presumably a result of their independent acquisition of hermaphroditism. Screening 7,500 haploid genomes, we have isolated one Fog mutant allele, *nm38*, and two Mog mutant alleles, *nm41* and *nm64*. *nm38* produces XX animals that are fully female and XO somatic males that make both sperm and then ooids. These phenotypes are similar to those produced in *C. elegans* by loss-of-function mutations in *Ce-fog-1* and *Ce-fog-3*. Surprisingly, however, *nm38* is not allelic to either of these genes' *C. briggsae* orthologs. Genetic mapping is in progress to determine the location of *nm38*. As judged by DIC microscopy and Hoechst staining of DNA, the germlines of *nm41* and *nm64* Mog mutant hermaphrodites sometimes contain excess mature sperm and spermatocytes reaching into their distal gonad arms; these animals never undergo oogenesis. Other homozygous *nm41* and *nm64* mutants have germline tumors. These phenotypes are similar to those produced by loss-of-function mutations in *Ce-atx-2* in *C. elegans* and by RNAi against *Cb-gld-1* in *C. briggsae*. We find by performing molecular candidate gene linkage assays that both *nm41* and *nm64* are tightly linked to *Cb-gld-1*, and direct sequencing of *nm41* has revealed a C/T transition that creates a stop codon in *Cb-gld-1* amino acid 14. Future work will include characterizing these and other mutant alleles recovered in forward genetic screens to investigate how their function and regulation compares to genes of the *C. elegans* germline sex determination pathway. Analyses of germline sex determination differences between *C. briggsae* and *C. elegans* will allow us to infer how hermaphroditism has independently evolved in this genus.

Evolution of the vulva intercellular signaling network in the *Caenorhabditis* genus

Marie-Anne Félix
(felix@ijm.jussieu.fr)

Address: Institut Jacques Monod, Tour 43, 2 pl. Jussieu,
75251 Paris Cedex 05, France

Abstract: The *Caenorhabditis* vulva is formed from a row of precursor cells in the ventral epidermis: P6.p normally adopts a central vulval fate (1°), P(5-7).p a lateral vulval fate (2°) and P(3,4,8).p a non-vulval fate (3°). This spatial pattern ('332123') is organized around the gonadal anchor cell (AC). The AC emits a LIN-3/EGF signal that induces the 1° fate in P6.p at high doses and can induce the 2° fate at low doses. Activation of EGF/Ras signaling in P6.p has two consequences: it induces the 1° fate in P6.p and activates a lateral Delta-Notch signaling pathway, which both induces the 2° fate and inhibits the 1° fate in P5.p and P7.p. by ablating the anchor cell at successive timepoints in the L3 stage, one uncovers a temporal series of P(5-7).p cell fate patterns, starting from '333' and ending with the correct '212' pattern, thus revealing the relative activities of different vulva signaling pathways. In *C. elegans* N2, the transition is rapid: only few other intermediate patterns are observed (Wang & Sternberg, unpublished), which suggests that P6.p, as soon as it is induced to a 1° fate by the AC, activates the 2° fate in P(5,7).p.

In *C. briggsae* AF16 and HK104, P(5-7).p display an intermediate '222' fate pattern: P6.p adopts a division pattern corresponding to mirror-image inner 2° fates (« vulCDDC »), confirmed using L4-stage expression of *mfIs5[Cb-egl-17::GFP]* and *mfIs8[Cb-zmp-1::GFP]*. Induction of the 1° fate thus requires a later or higher AC induction. Preliminary results suggest that the observed difference between *C. elegans* and *C. briggsae* is quantitative: the *C. elegans* situation can be reconstituted in *C. briggsae* by a mild LIN-3 overexpression, and conversely, the *briggsae* situation in *elegans* using a mild hypomorph of the Ras pathway.

In *C. remanei* PB4641 and PB228, the intermediate AC ablation results in a '232' pattern: P6.p adopts a 3° fate yet induces the 2° fate in P(5,7).p (these cells adopt a 3° fate upon simultaneous AC+P6.p ablations). Compared with *C. elegans*, this likely corresponds to changes in the relative weights of pathways downstream of Ras, with a high threshold for 1° fate induction and a low threshold for lateral signaling.

In *Caenorhabditis* species branching basally as well as in many other nematode genera, the intermediate '222' pattern is observed (ancestral 2-step induction). When these changes are mapped onto the *Caenorhabditis* phylogeny (Kiontke et al. PNAS 2004), the early specification of the 1° fate in P6.p appears between the *C.* sp. DF5070 and sp. 3

RGD1/PS1010 branches, and further evolution occurs in the *C. remanei* + *C. briggsae* branch. Intra-specific variations are detected within *C. japonica* and *C.* sp. 4 CB5161/PB2801 (for variations within *C. elegans*, see contribution by J. Milloz).

I will also present progress on using *Mos1*-based mutagenesis in *C. briggsae*. *Mos1* is a fly transposon that has been developed in *C. elegans* as a tag in mutagenesis (Bessereau et al. Nature 2001) and can now be used for gene replacement studies (Robert & Bessereau, IWM2005). *Mos1* transposition works in *C. briggsae*, although the frequency of transposition is so far low.

Molecular evolution of the AWA and AWC chemosensory pathways in the genus Caenorhabditis

Richard Jovelin
(rjovelin@uoregon.edu)

Authors: Richard Jovelin, R., and P.C. Phillips
Address: Center for Ecology and Evolutionary Biology
1210 University of Oregon, Eugene, OR 97403-1210 USA

Abstract: One of the fundamental questions in evolutionary genetics pertains to the understanding of the genetic basis of phenotypic variation. Chemosensation, analogous to taste and smell, is an important ecological trait in the genus Caenorhabditis and is the primary way these nematodes perceive their environment. Here we investigate the levels of nucleotidic variability for 6 genes within two chemosensory pathways mediated by the AWA and AWC neurons. We show that there is a little variability within the worldwide distributions of the primarily selfing *C. elegans* and *C. briggsae* while a single population of the outcrossing *C. remanei* exhibits higher degrees of polymorphism. Nevertheless, the difference in mating system is not enough to explain the differences in nucleotidic variability. Our data is consistent with the idea of a recent colonization by *C. elegans*, and suggest high rates of migration between populations coupled with strong purifying selection. Moreover, the coupling of patterns of molecular evolution with functional information reveals valuable insights about the structure and plasticity of these signaling pathways. We show that intraspecific polymorphism is equally distributed along the AWA and AWC pathways but that loci exhibit different levels of interspecific divergence. In particular we find that the G protein ODR-3 is under strong selection for maintained function in chemosensation and/or neuronal cilia development while the divergent transcription factor *odr-7* shows signatures of adaptive selection.

Duplication, divergence, and neofunctionalization of an Ikaros-like C2H2 zinc finger protein in *C. elegans*

Edward E. Large
(eelarge@ncsu.edu)

Authors: Edward E. Large[1], E.E., D.F. Kelleher[2], E.S. Haag[2], L.D. Mathies[1]
Address: [1] Department of Genetics, North Carolina State University, Raleigh, NC, U.S.A.
[2] Department of Biology, University of Maryland, College Park, MD, U.S.A.

Abstract: Gene duplication and divergence has long been recognized as a driving force of evolutionary change. Recent evidence suggests that, on average, genes are duplicated at the same frequency as the single nucleotide substitution rate (1). Furthermore, both humans and worms have higher duplication rates than *Drosophila*, *Arabidopsis*, or yeast (1). Although an estimated 30-60% of eukaryotic genes are the result of gene duplication, many have not been molecularly or functionally characterized. In this study we examine the duplication and divergence of the R08E3.4 family of C2H2 zinc finger genes in *C. briggsae (Cb)* and *C. elegans (Ce)*.

The four-cell gonad primordium is found in all nematodes and consists of two somatic gonadal precursors (SGPs: Z1/Z4) and two primordial germ cells (PGCs: Z2/Z3). In *Ce*, the SGPs are derived from mesodermal precursors during early embryogenesis and subsequently migrate to meet the PGCs midway through embryogenesis. The four cells of the gonad primordium do not divide again until the first larval stage. The vertebrate dHand ortholog, *hnd-1*, controls the early survival of the SGPs (2) whereas the vertebrate Gli ortholog, *tra-1*, controls the first division of the SGPs (3). The C2H2 zinc finger gene *ehn-3* interacts genetically with *hnd-1* to control survival of the SGPs (2) and with *tra-1* to control their first division (3). Surprisingly, *ehn-3* does not have an ortholog, but does possess a highly conserved paralog.

Preliminary results suggest *ehn-3* is one of four paralogs derived from the duplication of R08E3.4, a C2H2 zinc finger protein most similar to the Ikaros family of transcription factors. R08E3.4 is highly conserved in nematodes and has no clear paralogs in *Cb*. This provides the opportunity to understand the evolution of gene families and genetic

redundancy via the examination of R08E3.4 family members in *Ce* and *Cb*. We report the functional and molecular characterization of R08E3.4 family members in *Ce* and *Cb* and their relevant interactions.

(1) Lynch M, Conery JS. (2000) *Science* 290: 1151-55. (2) Mathies et al. (2003) *Development* Jul;130(13):2881-92.
(3) Mathies et al. (2004) *Development* 131, 4333-4343.

Evolution of vulval development within *C. elegans* species

Josselin Milloz
(milloz@ijm.jussieu.fr)

Authors: Milloz, J., I. Nuez, M.-A. Félix
Address: Intitut Jacques Monod, 75251 Paris Cedex 05, France

Abstract: Many organisms produce phenotypes that are robust to stochastic noise and to some environmental variations. This stability seems to arise from buffering properties of the underlying genetic networks that act during development. Importantly, such an environmental robustness may also result in a genetic robustness, thus allowing the evolution of developmental processes in the absence of phenotypic change (silent or cryptic evolution). We address this question using an extensively characterized model system: vulva development of *Caenorhabditis elegans*.

In the case of *C. elegans* vulva development, the final output (the cell fate pattern) is invariant within and among wild isolates. Out of 6 competent cells (P(3-8).p), only three cells normally adopt a vulval cell fate: a central fate (P6.p) and lateral fates (P5.p and P7.p). Buffering mechanisms and partial redundancies within and between the three molecular pathways involved (LET-60/Ras, LIN-12/Notch and BAR-1/b-Catenin) appear to guarantee a low degree of error. We want to explore the consequences of such a seemingly robust developmental system on the evolution of its underlying genetic network.

To 'debuffer' the system, and thus unravel potential cryptic genetic differences, we have introgressed mutations that affect the activities of the three pathways into six wild *C. elegans* isogenic backgrounds (JU258, CB4856, PB303, PB306, PS2025 and AB1). Our results show that the effect of a given mutation varies significantly between different genetic backgrounds. For the LET-60/Ras pathway, the mean number of induced cells ranges from 4.4 in N2 to 5.4 in PS2025 (n>50) with *let-60(n1046gf)*, and with *let-23(sy1rf)*, from 0.4 in JU258 to 2.2 in AB1 (n>50). Focusing on the N2 vs. CB4856 comparison, the number of induced cells is higher in CB4856 for all mutations affecting either the Ras or the Notch pathway. On the contrary, the defects due to an inactivation of the Wnt pathway (*bar-1(ga80lf)*) are enhanced in CB4856.

The variation in sensitivity to the same mutation between wild genetic backgrounds suggests an evolution affecting the vulva patterning network in the absence of any change in the final phenotype within the *C. elegans* species. We are now using i. cell ablation experiments and ii. signalling pathway activity quantification (using fluorescent reporter proteins, e.g. *plip-1*::YFP for Notch and *pegl-17*::CFP for Ras), to try and understand the basis of the differences unravelled by the mutation introgressions.

Wild worms: a window on ageing. Genotypic and phenotypic variation in lifespan in natural populations of *Caenorhabditis elegans*

Jeroen Pijpe
(pijpe@rulsfb.leidenuniv.nl)

Authors: Jeroen Pijpe[1,2], B.J. Zwaan[2], and J.E. Kammenga[1]
Address: [1]Laboratory of Nematology, Wageningen University
6709PD Wageningen, The Netherlands
[2]Leiden University, Institute of Biology, Section Evolutionary Biology,
2300RA Leiden, The Netherlands

Abstract: Ageing is the focus of medical and biological research, because ageing-related health problems are an increasing burden to human societies. The search for genetic pathways that regulate ageing has identified several. The most important pathway is the Insulin(-like)/IGF-1 Signalling pathway (IIS). Although a key regulator of ageing in mutant stocks in many species, it is by no means certain that this pathway underpins variation in ageing and lifespan in natural populations.

Mutationaly mapped genes have been obtained artificialy and are thus not connected to genes which have been selected in natural populations. This knowledge is crucial for the treatment of ageing-related diseases in humans, because it is within the scope of natural genetic variation that medical and non-medical treatments can be applied in human populations. However, the possibilities to study genetic mechanisms of ageing in human populations are very limited. The

strength of using *Caenorhabditis elegans* as an animal model is that its biology offers powerful genetic and genomic tools and fast experimental approaches that can significantly contribute to the elucidation of the ageing process, including (in humans).

My aim is to investigate whether genes in the Insulin(-like)/IGF-1 Signalling pathway contribute to natural variation in ageing. My approach is twofold. First, I will assess the phenotypic variation in ageing in existing natural populations of *Caenorhabditis elegans* divergent in lifespan. Secondly, for the most extremely long- and short lived populations, I will collect information on genetic variation in DNA sequence and gene-expression of IIS (-related) candidate genes for ageing. As such, I combine the extensive genomic and genetic knowledge of *C.elegans* with the relevant patterns of ageing that we see in natural populations to assess the significance of the role of the IIS in ageing for individual worms in the wild.

It is my intention to share my ideas and plans with the audience and invite them to give their opinion, as well as comments and suggestions on my proposal.

Evolution of somatic sex determination in nematodes.

André Pires da Silva
(apires@uta.edu)

Authors: Pires da Silva, A., and R.J. Sommer
Address: [1]Biology Dept. University of Texas at Arlington
Arlington, TX 76019-0498, USA
[2]Max-Planck-Institut für Entwicklungsbiologie
D-72076 Tübingen, Germany

Abstract: The *C. elegans* sex determination consists of a regulatory set of interactions that result in either the activation or inhibition of the gene *tra-1* in either sex. We are studying the molecular mechanisms by which this set of regulatory interactions has evolved. It has been hypothesized that sex determination evolved stepwise from the most downstream element (*tra-1*) by adding successively more upstream elements. Since the somatic sex determination is conserved in the genus *Caenorhabditis*, we decided to test this hypothesis by taking the more distantly related nematode *Pristionchus pacificus.* Importantly, *P. pacificus* has been recently established as a new genetic model system for which most tools necessary to clone mutants have been developed. Furthermore, the genomic sequence of this nematode has been become recently available. Using reverse genetics methods, we are systematically testing the function of homologs of *C. elegans* sex determination genes in *P. pacificus.* As a complementary and unbiased approach, we are also performing mutagenesis screens to isolate different kinds of *P. pacificus* sex determination mutants. Consistent with the proposed hypothesis, *tra-1* is conserved between *P. pacificus* and *C. elegans.* However, given the general observation that sex determination evolves rapidly in various phyla, we anticipate that the sex determination pathway will be different between these nematodes. The detected differences will be used to answer questions related to the genetic basis of evolutionary change.

Prediction of structured nonoding RNAs in the genomes of the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae*.

Dominic Rose
(dominic@bioinf.uni-leipzig.de)

Authors: Missal K., X. Zhu, D. Rose, W. Deng, G. Skogerbo, R. Chen, P.F. Stadler
Address: Department of Computer Science, University of Leipzig, Leipzig, Germany

Abstract: We present a survey for non-coding RNAs and other structured RNA motifs in the genomes of C. elegans and C. briggsae using the RNAz program. This approach explicitly evaluates comparative sequence information to detect stabilizing selection acting on RNA secondary structure. We detect 3672 structured RNA motifs, of which only 678 are known ncRNAs or clear homologs of known C. elegans ncRNAs. Most of these signals are located in introns or at a distance from known protein-coding genes. With an estimated false positive rate of about 50% and a sensitivity on the order of 50% we estimate that the nematode genomes contain between 3000 and 4000 RNAs with evolutionary conserved secondary structures. Only a small fraction of these belongs to the known RNA classes, including tRNAs, snoRNAs, snRNAs, or microRNAs. A relatively small class of ncRNA candidates is associated with previously observed RNA-specific upstream elements. About 60 novel ncRNAs resemble exhibit SMN binding sites and hence resemble snRNAs; these sequences have no detectable homologs outside the Nematodes.

Hypodermal endoreduplication and TGF-β drive adult body size and sexual size dimorphism in *C. elegans* and probably other nematodes.

Alberto Sáez

Authors: Saez, A.G.[1,2], E. Lozano[2], A. Flemming[2] , and A.M. Leroi[2]
Address: [1] Museo Nacional de Ciencias Naturales, CSIC, 28006 Madrid, Spain
[2] Imperial College London, Silwood Park Campus, Ascot, Berks SL57PY, UK

Abstract: How a nematode reaches its final body size? What is the basis for the differences in body size between its sexes (i.e. sexual size dimorphism or SSD)? We found that the answers for both questions hinge upon the levels of endoreduplication of the nuclei in the hypodermal syncitium (hyp7), which covers most of the worm and secretes its cuticle. First, the sizes of different species of nematodes, as well as their varying levels of SSD, positively correlate with their ploidy levels at hyp7. Second, using *C. elegans*, we have proven that correlation to be causal. We show that manipulating the levels of endoreduplication, both upwards and downwards, resulted in consistent changes in body size. We also found that null *dbl-1* (TGF-β) hermaphrodites diminish in size with respect to N2 hermaphrodites to a larger extent than males do. Therefore, *dbl-1* 31ot only accounts for final body size in worms, but for SSD. Finally, *dbl-1(0)* worms in which DNA synthesis was inhibited showed no significant further decline in size, thus suggesting that the control that *dbl-1* exerts over body size is via endoreduplication of hyp7.

Experimental co-evolution of *Caenorhabditis elegans* and its microparasite *Bacillus thuringiensis*

Rebecca Schulte
(becca@uni-muenster.de)

Authors: Schulte, R.D., C. Makus, B. Hasert, H. Schulenburg
Address: Department of Animal Evolutionary Ecology
University of Tuebingen, D-72076 Tuebingen, Germany

Abstract: The continuous interaction between a host and a parasite species result in extremely strong selection pressure on both partners. As soon as the host evolves an efficient defence, the parasite will alter infection and/or virulence mechanisms, which is again followed by the evolution of new host defences, potentially leading to a co-evolutionary arms race (Red Queen hypothesis). Thus, parasite-host co-evolution should be characterised by highly dynamic changes over time, which are likely to affect a diversity of life-history traits (in addition to defence and virulence). However, due to the scarcity of suitable experimental systems, the underlying mechanisms and resulting consequences are largely unknown. For this reason, we performed a laboratory-based selection experiment, in which the nematode *Caenorhabditis elegans* is allowed to interact with its microparasite *Bacillus thuringiensis* at different levels for about 50 host generations. In particular, we compared hosts that co-evolve with parasites, hosts that can adapt to a non-evolving parasite and hosts in the presence of a non-pathogenic control bacterium. By phenotypic and molecular analyses, we expect to obtain novel insights into the evolution of host defence and parasite virulence. We will specifically address the importance of specificity of defence/virulence, factors that enhance evolutionary change (outcrossing, recombination, mutation), life-history trade-offs (defence versus reproductive rate; virulence versus replication rate) and also the molecular genetic basis of defence and virulence.

The nature of selection for and against males in *Caenorhabditis elegans*

Henrique Teotónio

Authors: Teotónio, H.[1], D. Manoel[1], S. Carvalho[1], and P.C. Phillips[2]
Address: [1]Instituto Gulbenkian de Ciência, Apartado 14, 2780 Oeiras, Portugal
[2]Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, USA

Abstract: The presence of males in inbred lines of *C. elegans* is known to be rare under several laboratory environments, suggesting that outcrossing is selected against in this species. In two different studies we assess the role outbreeding among different inbred lines may have and also if the cost of males can be diminished when populations face higher than normal mutational loads. We underwent a 30 generation evolution experiment in a constant environment at high population sizes, where hybrids among several inbred lines were able to keep male frequency at approximately 10%. This response was correlated with the evolution of both higher male mating performance and hermaphrodite fecundity. In a second study we determined the consequences that an increase in the mutational load of four different isogenic wild isolates have for offsetting the selective cost of males. This was done by observing the frequency of males in androdioecious lines after ten generations of exposure to external mutagens. We found that the accumulation of deleterious mutations diminishes the cost of males. Further, we determined the viability effects of a single round of mutagen exposure and found that outcrossing completely overcomes the deleterious effects generated. Taken together our results support the view that male phenotypes in *C. elegans* can be under the influence of natural selection.

An evolutionary approach to identify the functional component of the *C. elegans* transcriptome

Itai Yanai
(yanai@mcb.harvard.edu)

Authors: Yanai, I., and C.P. Hunter
Address: Harvard Biological Laboratories, 16 Divinity Ave.
Cambridge, MA 02138, USA.

Abstract: A gene's pattern of expression is generally assumed to correlate to its function. However, recent analysis of whole-genome mRNA expression profiles collected from precisely staged *C. elegans* embryos challenges this fundamental assumption, suggesting that in addition to the transcription required for a gene to carry out its function, a gene may be expressed in other instances. Thus, a potentially sizeable fraction of the transcriptome may be selectively neutral. In this talk I will present our simple test of this idea as well as a general method for identifying probable neutral transcription through comparative transcriptomics. Our assumption is that non-functional expression will drift neutrally over evolutionary time, while functional/selected expression will be stable. This approach is analogous to comparing homologous gene sequences from divergent species. The method involves comparing, for each *C. elegans* gene, the effect of two evolutionary time frames: micro-evolution through a comparison of wild isolates and macro-evolution through a comparison of three Caenorhabditis species (*C. elegans*, *C. briggsae*, and *C. remanei*). Early embryonic development of the divergent species is phenotypically indistinguishable (showing very strong functional selection), yet their genomes have evolved to a tremendous degree. We are taking advantage of commercially available custom-design gene chip synthesis capability to compare the transcriptional programs for each, as well as testing predicted modes of evolution using reverse genetics and transgenic strains for a set of interesting instances of conservation and divergences in gene expression.

PARTICIPANT LIST

Michael **Ailion**
(michael.ailion@gmail.com)
University of Utah
Department of Biology, 257 S 1400 E
Salt Lake City, UT 84112 USA

Ricardo **Azevedo**
(razevedo@uh.edu)
Dept. Biology and Biochemistry,
University of Houston, Houston,
TX 77204-5001, USA

Scott **Baird**
(scott.baird@wright.edu)
Department of Biological Sciences
Wright State University
Dayton OH 45435-0001, USA

Antoine **Barrière**
(barriere@ijm.jussieu.fr)
Institut Jacques Monod, Tour 43, 2
pl. Jussieu, 75251 Paris Cedex 05, France

Gilberto Neves **Bento**
(gilberto.bento@tuebingen.mpg.de)
Abteilung IV(Evolutionsbiologie)
Max-Planck-Institut für Entwicklungsbiologie
Spemannstr. 35/I
D-72076 Tübingen, Germany

Andrea **Betancourt**
(abetanco@ed.ac.uk)
Institute of Evolutionary Biology
University of Edinburgh
King's Buildings, W. Mains Rd.
Ashworth Labs, Room 123
Edinburgh, EH9 3JT, UK

Christian **Braendle**
(braendle@ijm.jussieu.fr)
Institut Jacques Monod
Tour 43, 2 place Jussieu
75251 Paris cedex 05, France

Michael **Brauchle**
(mab404@nyu.edu)
Department of Biology,
New York University,
1009 Main Building,
100 Washington Square East,
New York, NY 10003-6688, USA

Dan **Bumbarger**
(bumbad01@student.ucr.edu)
Departments of Biology and Nematology,
University of California,
Riverside, CA 92507, USA

Sara **Carvalho**
(saracarvalho@igc.gulbenkian.pt)
Instituto Gulbenkian de Ciência
Apartado 14, 2780 Oeiras, Portugal

Helen M. **Chamberlin**
(chamberlin.27@osu.edu)
Department of Molecular Genetics
938 Biological Sciences Building
484 West 12th Avenue
Ohio State University
Columbus, OH 43210, USA

Chris **Chandler**
(cholden@iastate.edu)
253 Bessey Hall
Department of Ecology, Evolution,
and Organismal Biology
Iowa State University
Ames, IA 50011-1020 USA

Audrey **Chang**
(audrey.chang@duke.edu)
Biology Department
Duke University
Research Drive, LSRC B333
Box 91000, Durham, NC 27708 USA

King L. **Chow**
(bokchow@ust.hk)
Department of Biology,
Bioengineering Program,
Hong Kong University of Science and
Technology, Clear Water Bay,
Kowloon, Hong Kong, China

Avril **Coghlan**
(alc@sanger.ac.uk)
Wellcome Trust Sanger Institute,
Cambridge, UK.

Asher D. **Cutter**
(asher.cutter@ed.ac.uk)
Institute of Evolutionary Biology
University of Edinburgh
King's Buildings, W. Mains Rd.
Ashworth Labs, Room 123
Edinburgh, EH9 3JT, UK

Marie **Delattre**
(marie.delattre@isrec.ch)
ISREC- chemin des boveresses, 155
1066 Epalinges/Lausanne, Switzerland

Margarida **Delgado**
(mxdelgado@isa.utl.pt)
Sec. Genética - CBAA
Instituto Superior de Agronomia
Lisboa, Portugal

Dee R. **Denver**
(denvedee@cgrb.oregonstate.edu)
Department of Zoology
Center for Genome Research and
Biocomputing, Oregon State University
2000 Cordley Hall, Corvallis, OR 97331 USA

Alivia **Dey**
(Dey@Biology.Rutgers.Edu)
Rutgers University,
Nelson Biological Labs (rm B326),
604 Allison Rd., Piscataway,
NJ 08854-8082, USA.

Anaid **Diaz**
(s.diaz-palacios.1@research.gla.ac.uk)
Rm 303, Graham Kerr Bldg.
Theoretical Ecology Group,
Division of Environmental &
Evolutionary Biology, University of Glasgow,
Glasgow G12 8QQ, UK

Elie **Dolgin**
(elie.dolgin@ed.ac.uk)
Institute of Evolutionary Biology
University of Edinburgh
Ashworth Laboratories, King's Buildings,
West Mains Road, Edinburgh EH9 3JT, UK

Agnieszka **Doroszuk**
(Agnieszka.Doroszuk@wur.nl)
Laboratory of Nematology
Wageningen University, Binnenhaven 5
6709 PD Wageningen, The Netherlands

Alana **Doty**
(adoty@umd.edu)
Department of Biology,
University of Maryland,
College Park, MD, U.S.A.

Ronald **Ellis**
(ellisre@umdnj.edu)
The UMDNJ School of Osteopathic Medicine,
B303 Science Center
Two Medical Center Drive
Stratford, NJ 08084, USA

Scott W. **Emmons**
(emmons@aecom.yu.edu)
Department of Molecular Genetics
Albert Einstein College of Medicine
1300 Morris Park Ave., Bronx, NY 10461,
718-430-3130, USA

Marie-Anne **Félix**
(felix@ijm.jussieu.fr)
Institut Jacques Monod, Tour 43,
2 pl. Jussieu, 75251 Paris Cedex 05, France

David H.A. **Fitch**
(david.fitch@nyu.edu)
Department of Biology
New York University
Main Building, Room 1009,
100 Washington Square East, New Yor
NY 10003, USA

Bhagwati P. **Gupta**
(guptab@mcmaster.ca)
McMaster University
Hamilton, ON L8S 4K1, Canada

Eric **Haag**
(ehaag@umd.edu)
Department of Biology
0256 Biology/Psychology Building
University of Maryland
College Park, MD 20742, USA.

Simon **Harvey**
(Simon.Harvey@bristol.ac.uk)
School of Biological Sciences,
University of Bristol Woodland Road,
Bristol BS8 1UG, UK

Jody **Hey**
(Hey@Biology.Rutgers.Edu)
Department of Genetics, Rutgers University,
Nelson Biological Labs (rm B326),
604 Allison Rd. Piscataway,
NJ 08854-8082, USA

Richard **Jovelin**
(rjovelin@uoregon.edu)
Center for Ecology and Evolutionary Biology,
1210 University of Oregon
Eugene, OR 97403-1210 USA

Jan E. **Kammenga**
(Jan.Kammenga@wur.nl)
Laboratory of Nematology
Wageningen University, Binnenhaven 5
6709 PD Wageningen, The Netherlands

Karin **Kiontke**
(kk52@nyu.edu)
Department of Biology
New York University
Main Building, Room 1009,
100 Washington Square East, New York,
NY 10003, USA

Dan **Koboldt**
(dkoboldt@genetics.wustl.edu)
Washington University School of Medicine,
4566 Scott Avenue, Box 8232
St. Louis, Missouri 63110, USA

Edward E. **Large**
(eelarge@ncsu.edu)
Department of Genetics,
North Carolina State University,
Raleigh, NC, U.S.A.

Encarni **Lozano**
(e.lozano@imperial.ac.uk)
Depto. de Biodiversidad y
Biologia Evolutiva, Museo Nacional de
Ciencias Naturales, CSIC, Jose Gutierrez
Abascal, 2. 28006 Madrid, Spain

Diogo **Manoel**
(diogomanoel@igc.gulbenkian.pt)
Instituto Gulbenkian de Ciência
Apartado 14, 2780 Oeiras, Portugal

João **Marchante**
(jmarchante@gmail.com)
Instituto Superior de Agronomia
Lisboa, Portugal

Laura **Mathies**
(ldmathies@ncsu.edu)
3513 Gardner Hall
North Carolina State University
Raleigh, NC 27695-7614, USA

Josselin **Milloz**
(milloz@ijm.jussieu.fr)
Institut Jacques Monod
2 pl. Jussieu, tower 43
75251 Paris Cedex 05, France

Andy **Peters**
(adpeters1@wisc.edu)
Department of Zoology
Birge Hall, 430 Lincoln Dr.
University of Wisconsin
Madison, WI 53706, USA

Patrick C. **Phillips**
(pphil@uoregon.edu)
Center for Ecology and
Evolutionary Biology
5289 University of Oregon,
Eugene, OR 97403-5289 USA

Jeroen **Pijpe**
(pijpe@rulsfb.leidenuniv.nl)
Laboratory of Nematology
Wageningen University, Binnenhaven 5
6709 PD Wageningen, The Netherlands

Dave **Pilgrim**
(dave.pilgrim@ualberta.ca)
BS G 507 Biological Sciences Building
Department of Biological Sciences
University of Alberta
Edmonton, Alberta
CANADA T6G 2E9

André **Pires da Silva**
(apires@uta.edu)
501 S. Nedderman, 337 Life Science
Building, Biology Dept (Box 19498)
University of Texas at Arlington
Arlington, TX 76019-0498, USA

Matthew V. **Rockman**
(mrockman@princeton.edu)
Lewis-Sigler Institute for Integrative
Genomics, 110 Carl Icahn Labs,
Princeton University
Princeton, NJ 08544, USA

Dominic **Rose**
(dominic@bioinf.uni-leipzig.de)
University of Leipzig
Department of Computer Science
Haertelstr. 16-18, Postal code: D-04107
Leipzig, Germany

Alberto G. **Sáez**
(asaez@mncn.csic.es)
Depto. de Biodiversidad y
Biologia Evolutiva, Museo Nacional de
Ciencias Naturales, CSIC, Jose Gutierrez
Abascal, 2. 28006 Madrid, Spain

Matthew P. **Salomon**
(msalomon@zoo.ufl.edu)
Department of Zoology
P. O. Box 118525
University of Florida
Gainesville, FL 32611-8525, USA

Hinrich **Schulenburg**
(hinrich.schulenburg@uni-tuebingen.de)
Department of Animal Evolutionary
Ecology, Zoological Institute,
University of Tuebingen,
Auf der Morgenstelle 28
72076 Tuebingen, Germany

Rebecca **Schulte**
(becca@uni-muenster.de)
Department of Animal Evolutionary
Ecology, Zoological Institute,
University of Tuebingen,
Auf der Morgenstelle 28
72076 Tuebingen, Germany

Erich **Schwarz**
(emsch@its.caltech.edu)
Division of Biology, 156-29,
California Institute of Technology,
Pasadena, CA, 91125, U.S.A.

Henrique **Teotónio**
(teotonio@igc.gulbenkian.pt)
Instituto Gulbenkian de Ciência
Apartado 14, 2780 Oeiras, Portugal

W. Kelley **Thomas**
(kelley.thomas@unh.edu)
Hubbard Center for Genome Studies
University of New Hampshire
35 Colovos Road, 4th Floor,
Durham, NH 03824, USA

Itai **Yanai**
(yanai@mcb.harvard.edu)
Harvard Biological Laboratories
16 Divinity Ave.
Cambridge, MA 02138, USA.

Greta Martins
(Admnistrative Support, IGC)