

II Pea Aphid Genome Annotation Workshop

4th - 5th of June
Centre for Genomic Regulation
Barcelona, Spain



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FOREWORD

Foreword

“I am beginning to construct an aphid resources web page. I have begun by providing links to web sites that contain useful information on aphid genomics. If you would like me to link your web site (..) Cheers, David Stern” - this is an extract from the first e-mail available in the archives of the aphidgenomics mailing list at Princeton University. It was written in July 2003 at a time in which the white paper¹ proposing the sequencing of the Pea Aphid *Acyrtosyphon pisum* was being written. This species was chosen as a model for aphids, an important insect group that comprises agronomical pests that can act as vectors of plant viruses. Moreover, the aphids provide us with an excellent model system to study many important biological questions related with development, ecology and evolution. Thanks to the effort of the International Aphid Genomics Consortium (IAGC) steering committee², the proposal was eventually accepted for funding by NHGRI (March 2005) and finally assigned to the sequencing center at the Baylor College of Medicine (May 2006). Then, the genomic sequences of this organisms started to flow.

After the completion of the *Acyrtosyphon pisum* genomic sequence (soon to be published), aphid research has finally entered the genome era. The usefulness of this resource is already evident by the many relevant new biological insights that are reported in the main paper and the long list of companion publications. Datasets and tools related to the genome sequences are also being generated: EST collections, micro-array and other transcriptomics analyses, proteomics analyses, metabolomics, the pea aphid phylome, etc. This wealth of genomic data will certainly drive much of the research on aphids from now on. Therefore, the work does not end up with the completion of the genome sequence, it rather starts now. This two-days workshop will gather researchers from all over the world who will discuss recent advances and future prospects of this emerging field. Hopefully this meeting will create the perfect environment for discussions on possible sequencing of additional aphid sequences, opportunities offered by new technologies, ideas for other joint community efforts, etc. Finally, I also hope you enjoy your stay in Barcelona, both scientifically and personally. The CRG and the local organizing committee is at your disposal to make your stay a very fruitful and pleasant one.

Best wishes,

Toni Gabaldón

1. <http://www.hgsc.bcm.tmc.edu/documents/PeaAphidGenomeWhitePaper.pdf>
2. In alphabetical order, and in bold those attending this workshop: *Marina Caillaud, Owain Edwards, Linda Field, Danièle Giblot-Ducray, Stewart Gray, David Hawthorne, Wayne Hunter, Georg Jander, Nancy Moran, Andres Moya, Atsushi Nakabachi, Hugh Robertson, Kevin Shufran, Jean-Christophe Simon, David Stern, Denis Tagu*
3. See press release at: <http://www.genome.gov/13014443>



COMMITTEES

Organizing Committee

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Denis Tagu.
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Alex Wilson.
Miami University, USA

James Carolan.
University College Dublin, Ireland

Owain Edwards.
CSIRO. Australia

Atsushi Nakabachi.
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Local Organizing Committee

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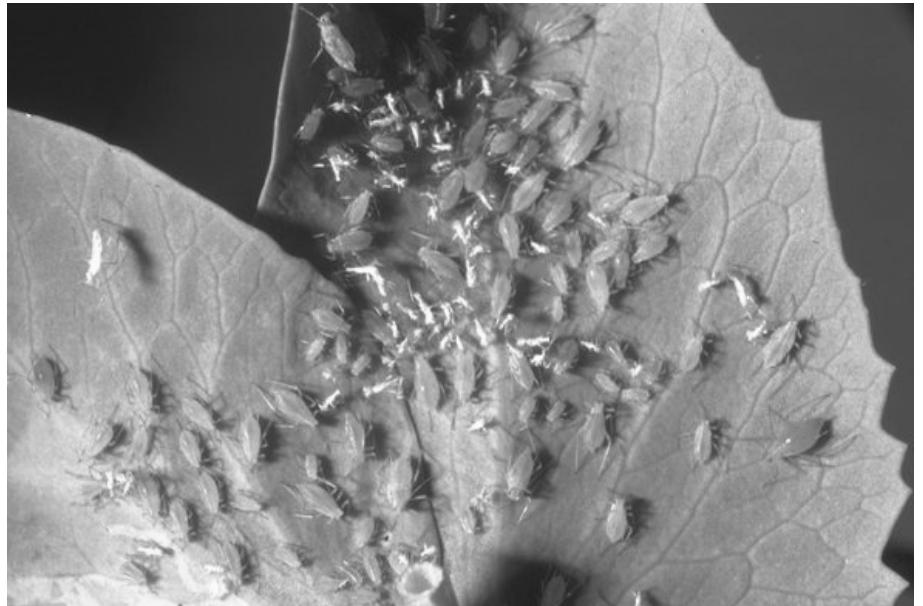
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Diego Kormes.
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SCIENTIFIC PROGRAMME

Wednesday 3rd June 2009

17:00 - 19:00: Early registration. (Reception Desktop PRBB Building)

Thursday 4th June 2009

09:00 - 09:45h Registration. (Reception Desktop PRBB Building)

09:30 - 10:00h Introductions. (Room Aula 473. 4th Floor)

10:00 - 11:30h First session “Genome annotation”.
Chair: Owain Edwards. (Room Aula 473. 4th Floor)

10:00 - 10:30 James Carolan. “Assisting Functional Annotation with Proteomics and Mass Spectrometry”

10:30 - 11:00 Fabrice Legeai. “AphidBase : feedback on the genome annotation, new tools and perspectives.”

11:00 - 11:30 Toni Gabaldón. “Towards the use of large-scale phylogenetics to assist in the annotation of newly sequenced genomes: the *Acyrtosiphon pisum* genome sets a precedent”

11:30 - 12:00h Coffee break. (Terrace. 5th Floor)

12:00 - 13:00h Second session “Metabolism”.
Chair: Toni Gabaldón. (Room Aula 473. 4th Floor)

12:00 - 12:30 Sandy MacDonald “The power of combining genome annotation and systems level modeling”

12:30 - 13:00 Stefano Colella. “The *Acyrtosiphon pisum* Cyc database (AcypiCyc) created using a novel BioCyc Annotation Database System (CycADS): a useful tool to explore and study the pea aphid metabolism.”

13:00 - 15:00h Lunch. (Terrace. 5th Floor)

15:30 - 17:00h Collaborative groups sessions:

GROUP A: Proteomics group. Chair: James Carolan. (Room 470 Sem 1. 4th floor)

GROUP B: Transcriptomics. Chair: Alex Wilson. (Room 468 Sem 2. 4th floor)

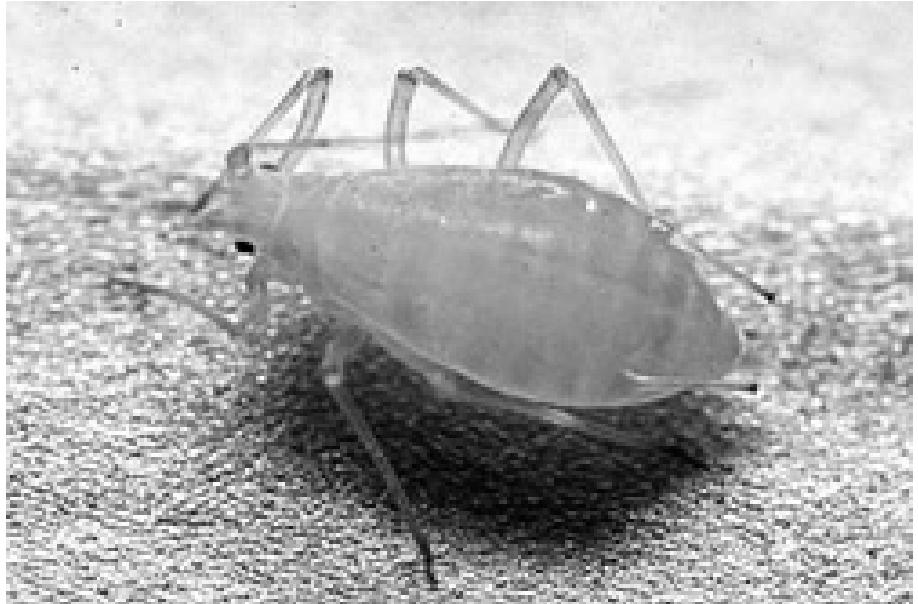
GROUP C: Training troubleshooting session I on aphid genomics related resources:
Apolo, AphidBase, AcypiCyc, PhylomeDB, etc.
(Room 635.02 Alt Sud. Entry by the 5th floor)

17:00 - 19:00h Coffee break and Poster Session
(Room 468-470 Sem 1/2. 4th floor)

21:00h Dinner at the Arenal Restaurant (<http://www.arenalrestaurant.com/>)

Friday 5th June 2009

09:30 - 11:00h	Third session “Biological processes in the pea aphid I” Chair: Yvan Rahbé. (Room 468-470 Sem 1/2. 4 th floor)
09:30 - 10:00	Filipe Vieira. “Comparative genomic analyses of the odorant-binding proteins in the pea aphid <i>Acyrtosiphon pisum</i> ”
10:00 - 10:30	Chun-che Chang. “Cloning and developmental analysis of germline toolkit genes in the parthenogenetic pea aphid <i>Acyrtosiphon pisum</i> ”
10:30 - 11:00	Daniel Price. “The functional role of <i>A.pisum</i> sugar transporter 1 (<i>ApST1</i>) from the aphid gut.”
11:00 - 11:30h	Coffee break. (Terrace. 5 th Floor)
11:30 - 14:00h	Workshops of collaborative groups and trainig troubleshooting session
Group D:	Salivary secretome and proteases group. Chairs: O. Edwards and J. Carolan. (Room 470 Sem 1. 4 th floor)
Group E:	Training troubleshooting session II on aphid genomics related resources: Apolo, AphidBase, AcypiCyc, PhylomeDB, etc. (Room 635.02 Alt Sud. Entry by the 5 th floor)
14:00 - 15:30h	Lunch. (Terrace. 5 th Floor)
15:30 - 16:30h	Fourth session “Biological processes in the pea aphid II” Chair: James Carolan. (Room 468-470 Sem 1/2. 4 th floor)
15:30 - 16:00	Tom Walsh. “DNA methylation in the pea aphid <i>Acyrtosiphon pisum</i> .”
16:00 - 16:30	Owain Edwards “Identifying and characterizing the aphid salivary secretome”
16:30 - 17:00h	Coffee break. (Terrace. 5 th Floor)
17:00 - 18:00h	Virtual session: Videoconferences. Chair: Toni Gabaldón. (Room 468-470 Sem 1/2. 4 th floor)
17:00 - 17:20	Denis Tagu “Using chemical mutagenesis for forward genetics on the pea aphid: utopia or reality?”
17:20 - 17:40	Stephen Richards “Update on the pea aphid genome sequencing”
17:40 - 18:00	Justin Pachebat “Happy mapping”
18:00 - 19:30h	IAGC organization, general discussion and concluding remarks. Chair: Alex Wilson. (Room 468-470 Sem 1/2. 4 th floor)



ABSTRACTS

trimAl: A tool for automated alignment trimming.

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Abstract

Multiple sequence alignments (MSA) are central to many areas of bioinformatics, including phylogenetics, homology modeling, database searches and motif finding. Recently, such MSA-based techniques have been incorporated in high-throughput pipelines such as genome annotation and phylogenomics analyses. In all these applications the reliability and accuracy of the analyses depend critically on the quality of the underlying alignments. A plethora of computer programs and algorithms for MSA are currently available (Notredame 2007) that implement different heuristics to find mathematically optimal solutions to the MSA problem. Accuracies of 80-90% have been reported for the best algorithms, but even the best-scoring alignment algorithms may fail with certain protein families or at specific regions in the alignment.

It is therefore generally assumed that trimming the alignment, so that poorly aligned regions are eliminated, increases the accuracy of the resulting MSA-based applications (Talavera and Castresana 2007). Some programs such as G-blocks (Castresana 2000) have been developed to assist in the MSA trimming phase by selecting blocks of conserved regions. However, their use over larger datasets is hampered by the need for defining, prior to the analysis, the set of parameters that will be used for all sequence families. Here we present trimAl, a tool for automated alignment trimming. The possibility for automatically adjusting the parameters to improve the phylogenetic signal-to-noise ratio, makes trimAl especially suited for large-scale phylogenomic analyses, involving thousands of large alignments.

Literature cited

- Notredame, C. (2007), Recent evolutions of multiple sequence alignment algorithms, PLoS Comput Biol, 3 (8), e123.
- Talavera, G., Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments, Syst Biol, 56 (4), 564-77.
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol, 17 (4), 540-52.
- Huerta-Cepas, J., et al. (2008), PhylomeDB: a database for genome-wide collections of gene phylogenies, Nucleic Acids Res, 36 (Database issue), D491-6.

Assisting Functional Annotation with Proteomics and Mass Spectrometry

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Abstract

As we enter the systems biology age of aphids the impending application of the various disciplines will undoubtedly yield fascinating insights into the biology of these extraordinary insects. Throughout our community, transcriptomics, proteomics, metabolomics and epigenomics are already yielding significant biological returns from even the most preliminary of applications. Central to the advent of aphid systems biology has been the near completion of the pea aphid genome and subsequent gene annotation. The power afforded by the availability of the genome is clearly evident with respect to proteomics. Lists of *in silico* tryptic peptides generated from gene sequences, and the estimated masses of these peptides and their fragmented component amino acids are used as a reference to match and identify the peptides being analysed by mass spectrometry. Thus our ability to perform proteomic analyses has clearly benefited by the availability and development of this fantastic resource.

Can proteomics return the favour? A proteome catalogue of a particular fraction or tissue is not simply a list of proteins – it holds validation for the coding status of all proteins (and genes) identified and yields spatial information relating to gene expression. The function and significance of orphan/novel proteins has already benefited from the determination of peptide abundance in particular fractions. Identified peptides offer support for one isoform (or splice variant) over another. Differential analyses (using 2DE or isotope tagging) provide a view of whole organism proteome change, yielding information about expression changes of individual proteins.

As we enter the functional annotation stage of this sequencing project the next challenge is to manage this valuable information and ensure that it is utilised fully. As more groups apply proteomics and mass spectrometry to aphid biology the opportunity to develop an open and community-based proteomic resource presents itself. The establishment of an Aphid Proteomics Group will result in the collation of existing and future protein lists, 2D gel images, mass spectra etc. into repositories, yielding a resource open to those interested in aphid biology. In addition suitable laboratories and groups could be identified to conduct the future proteomic research plans of the IAGC. If the aim of the IAGC “is to develop the aphid model system to the same level of molecular, cell, and developmental biological understanding as other model insects” our concerted efforts in the field of proteomics is required.

The *Acyrthosiphon pisum* Cyc database (AcypiCyc) created using a novel BioCyc Annotation Database System (CycADS): a useful tool to explore and study the pea aphid metabolism.

Stefano Colella^{1,3,‡}, Augusto Vellozo^{1,2,3,‡}, Ludovic Cottret^{2,3}, Gérard Febvay^{1,3}, Federica Calevro^{1,3}, Yvan Rahbé^{1,3}, Angela Douglas⁴, Marie-France Sagot^{2,3} and Hubert Charles^{1,3}

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‡ These authors contributed equally to this work

Abstract

The availability of the genome sequence of the pea aphid (*Acyrthosiphon pisum*), together with the already available sequence of its primary symbiont (*Buchnera*) genome, opens the way to the use of global systems biology approaches to study its metabolism. To perform such analyses the most updated annotation data have to be collected in a standard format and updated regularly. To this end we developed AcypiCyc, a BioCyc database dedicated to the pea aphid that in its present version includes also the metabolic annotation for its bacterial symbiont and for *Drosophila melanogaster*.

We have also developed CycADS (Cyc Annotation Database System): an automated annotation management system to allow the integration of the latest sequence information into the metabolic network reconstruction and analysis. CycADS is centered on an *ad hoc* SQL database, complemented by a set of Java scripts to import and export relevant information. Data from GeneBank and from different gene annotation tools (such as KAAS, BLAST2GO, etc) are collected into the database and later extracted, using a score based filter system, to generate a complete input file to build and/or update AcypiCyc using the ‘Pathway tools’ software (BioCyc). The CycADS pipeline will allow an easy update of the AcipiCyc database over time.

All genes annotated are present in the database: different query tools allow the users to visualize different metabolic reactions or pathways and metabolism comparisons tools are also available. All pages are complemented with hyperlinks to different information resources including genomics (AphidBASE and GeneBank), phylogenomics (PhylomeDB) and metabolism (KEGG orthology, BRENDA, ENZYME) databases.

AcypiCyc offers a framework for the integrated analysis of metabolic networks and the possibility to browse the pea aphid metabolism. Thanks to its open platform formats (BioPAX, SBML), the data can be integrated into other tools to perform both metabolic network and complex genomics data analyses.

URLs:

<http://pbil.univ-lyon1.fr/software/cycads/acypicyc/home>

Identifying and characterizing the aphid salivary secretome

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Abstract

The primary interface between an aphid and its host plant is its saliva. Gelling saliva provides a barrier at the entry point of the stylets into the leaf, and surrounds the stylets in a sheath as they move between cells towards the vascular bundle. Watery saliva, secreted before and throughout feeding, is thought to condition the plant for successful feeding, but the specific functions of the watery saliva remain poorly understood. There is growing evidence that successful plant defence against aphids is mediated by the same mechanisms that plants use to defend against pathogen attack. It is therefore conceivable that the secreted proteins in aphid saliva, the “secretome”, may have homologous functions to the effector proteins secreted into plants by pathogens.

In recent years, due in large part to the availability of the pea aphid (*Acyrtosiphon pisum*) genome sequence, there have been significant advances in identifying the proteins which make up the aphid salivary secretome. Proteomic and mass spectrometry approaches have been used to directly identify salivary proteins in two aphid species, the pea aphid and the green peach aphid (*Myzus persicae*) and generate a first pass catalogue of the pea aphid salivary gland proteome. Salivary EST libraries have been used to identify additional secretome candidates based on relative expression levels and secretion signals. Additional candidates may be identified using bioinformatics algorithms similar to those used to successfully identify secreted effectors in pathogen genomes. Much future work is needed to validate these candidates as secreted salivary proteins – including research in basic biology and behaviour, molecular biology, and biochemistry.

In order to most efficiently achieve our goal of identifying and characterizing the aphid salivary secretome, we are proposing to form an international consortium of scientists working collaboratively to achieve this ambitious goal. There is much international interest in characterizing pathogen effectors, not only to understand pathogenesis but also as tools to understand the fundamental aspects of cell biology that are often mimicked or disrupted by effector function. Our aphid model provides unique input to this global effort as it represents a new evolutionary group of “pathogens”, affording distinct experimental advantages over traditionally defined pathogens including: (1) the secretome is produced by a single tissue that is easily isolated, and (2) the saliva can be collected and isolated from an artificial medium. Characterising the secretome of aphids will not only afford insight into the plant-aphid interaction at the molecular level but as with other pathogens may lead to the discovery of potential targets to control this important pest.

Towards the use of large-scale phylogenetics to assist in the annotation of newly sequenced genomes: the *Acyrtosiphon pisum* genome sets a precedent

Toni Gabaldón¹, Jaime Huerta-Cepas¹, Marina Marcet-Houben¹, Miguel Pignatelli²
and Andrés Moya²

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Abstract

The analysis of all gene phylogenies can be used to produce a set of highly-reliable predictions of orthology and paralogy relationships among the species considered (Gabaldón 2008; Huerta-Cepas et al. 2007). This application could be of particular relevance in the context of newly sequenced genomes, since it allows for reliable automated transfers of functional annotations based on clear orthology, rather than just homology, relationships. Moreover, the availability of phylogenetic trees and multiple sequence alignments can serve many other purposes such as the prediction of protein function or the detection of important evolutionary events. Therefore, we argue that the inclusion of large-scale phylogenetic analyses in the annotation pipelines of newly sequenced genomes will significantly increase its quality as well as provide an interesting data set to derive evolutionary and functional hypothesis of the species of interest. Within the context of the sequencing of the genome of the pea aphid *Acyrtosiphon pisum*, we undertook a phylogenetic analysis for every protein of this species. The resulting phylome includes the evolutionary relationships of all aphid proteins and their homologs among 13 other fully-sequenced arthropods and three out-group species. Here we will report some of the major findings and show how this information can be exploited with PhylomeDB (Huerta-Cepas et al. 2008).

Literature cited

- Gabaldón, T. 2008. Large-scale assignment of orthology: back to phylogenetics? *Genome Biol* **9**: 235.
- Huerta-Cepas, J., A. Bueno, J. Dopazo, and T. Gabaldón. 2008. PhylomeDB: a database for genome-wide collections of gene phylogenies. *Nucleic Acids Res* **36**: D491-496.
- Huerta-Cepas, J., H. Dopazo, J. Dopazo, and T. Gabaldón. 2007. The human phylome. *Genome Biol* **8**: R109.

AphidBase, a centralized database for aphid genes and genomes.

Jean-Pierre Gauthier, Fabrice Legeai and Denis Tagu.

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Abstract

Since the availability of different genomic resources on aphids developed by the International Aphid Genomics Consortium (IAGC), AphidBase, a database centre which aim is to permit easy access, analysis, annotation and display of aphid genomic data, has been launched (<http://www.aphidbase.com/>).

Based on an open source project called GMOD, the database schema (chado) is accessible via a web browser (Gbrowse) which allows visualisation of different evidences and predictions generated by the consortium members and where each detailed feature is linked to other sources of information, like detailed report, NCBI Entrez page, phylogenetic tree and a metabolic pathway.

The following features are displayed:

- 7 different gene predictors
- 169667 EST from *A. pisum*
- 30840 EST from other aphid species
- 189 miRNAs of *A. pisum*
- Transposable elements
- *Drosophila melanogaster* and *Apis mellifera* homologs
- Uniprot homologs

The first official reference gene set of *Acyrthosiphon pisum* is composed of 34,603 automatically predicted genes. 10248 of them are RefSeqs from the NCBI database Reference Sequences database and the others comes from Glean predictions.

These data, combined with the use of a BLAST service and the APOLLO annotation tool, have permitted the manual annotation of more than 2000 genes.

Beside this, AphidBase provides tools for sequence analysis and browsing such as a full text search engine, news and community links, download facilities and a Wiki.

Also included are the links to PhylomeDB, a comprehensive phylogenomic database for the pea aphid and AcypiCyc, a specialized database on the metabolic networks of aphids and their symbionts.

In conclusion, AphidBase is a very useful tool for all the community, allowing sharing and access to the data as well as providing tools for the *Acyrthosiphon pisum* genome annotation.

In the future we will need to incorporate and analyse the massive amount of data generated by New Generation Sequencing technologies and new functionalities like comparative genomics, advanced queries, complex gene report are to be introduced.

PhylomeDB 2.0: A database for complete collections of gene phylogenies and orthology and paralogy predictions

Jaime Huerta-Cepas, Salvador Capella-Gutierrez, Marina Marcet-Houben, Leszek Prysycz, Diego Kormes and Toni Gabaldón*.

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Abstract

PhylomeDB is a public database for complete collections of gene phylogenies (phylomes). It allows users to explore the evolutionary history of genes through the visualization of phylogenetic trees and alignments and to obtain their phylogeny-based orthology and paralogy relationships across a number of species. PhylomeDB aims at providing high-quality phylogenetic analyses of complete genomes. Thus, all the phylogenetic trees hosted in phylomeDB were obtained by using some of the most accurate methodologies to date, such the Maximum Likelihood or Bayesian approaches. Most phylomes include also evolutionary model tests for each gene phylogeny. Moreover, alignments in which these phylogenies are based were reconstructed by iterative methods that include a refinement phase, processed to remove poorly aligned regions. Finally, orthology and paralogy relationships provided by PhylomeDB are always based on the analysis of gene phylogenies, which account better for the complexity of such relationships in intricate gene families.

The pea aphid phylome (internally registered in phylomeDB with code '16') comprises a total of 23,523 Maximum Likelihood gene phylogenies and multiple sequence alignments. These data can easily be browsed through the main search panel at the phylomeDB web interface or downloaded at convenience. Both RefSeq and AphidBase gene identifiers are supported for querying PhylomeDB entries. Additionally, a BLAST-based sequence search may help users finding their proteins of interest. Aphid related databases such as AphidBase (<http://www.aphidbase.com>) and AcypiCyc (<http://acypicyc.cycadsys.org>) already link their data to the evolutionary information stored in phylomeDB.

PhylomeDB can be publicly accessed at <http://phylomedb.org>

AphidBase: feedback on the genome annotation, new tools and perspectives

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Abstract

AphidBase (<http://www.aphidbase.com>) is a centralized bioinformatic resource that was developed to facilitate community annotation of the pea aphid genome by the International Aphid Genomics Consortium (IAGC). The system includes Apollo and GBrowse utilities as well as a wiki, blast search capabilities and a full text search engine. These tools were used by a large and dispersed international community to appraise more than 2 thousands gene models.

In summary, about 28% of the appraised predictions needed correction. Curators predominantly investigated genes with at least some biological evidence and similarity with known proteins (i.e. RefSeq genes). 80% of predicted genes with no evidence (i.e. Glean genes) were corrected by hand while only 19% of the RefSeq genes.

The development of new AphidBase Information System is still undergoing, in particular a new gene report with full functionalities has been very recently set up. It offers many information about a gene, and its transcripts and peptides, functionnal annotation, environment and expression level, at a glance. We are also upgrading the search tool with the capability of searching with any term (keyword, gene name, identifiers accession number, domain, Gene ontology term or id etc...).

Finally, we will discuss how AphidBase would help to handle the millions of short reads Next Generation Sequencing technologies, especially for re-annotation, expression profiling (RNA-Seq), variability study or ChIP-Seq.

Cloning and developmental analysis of germline toolkit genes in the parthenogenetic pea aphid *Acyrtosiphon pisum*

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Abstract

Germ cells are specified during embryogenesis in most animals. In some species germline specification occurs during early development, depending on maternal germline determinants in the germ plasm; in other species specification of germ cells is induced by signals released from neighboring somatic cells. Interestingly, it has been demonstrated that no matter how germ cells are specified, animals utilize common genes—the “germline toolkit genes” (GTGs), for the development of germ cells. In the genome of the pea aphid genome *Acyrtosiphon pisum* we have identified and cloned twenty-one GTGs, including the most conserved GTGs: *vasa* and *nanos*. Interestingly, gene duplication has occurred to both *vasa* and *nanos*—we have identified four homologues each of *vasa* and *nanos* in the pea aphid. Whole-mount *in situ* hybridization results show that one *vasa* (*Apvasa1*) and two *nanos* (*Apnanos1* and *Apnanos2*) genes are germline specific but expression of *Apvasa2-4* and *Apnanos3-4* are not restricted to embryonic germ cells. At present we are investigating the distribution of gene products encoded by all *Apvasa* and *Apnanos* genes. In particular, we aim to explore how translation control works on transcripts that are not specifically expressed in the germ cells. Future experiments include the developmental and functional analysis of all GTGs in both sexual and sexual pea aphids in order to understand the evolutionary and developmental roles of GTGs and how they regulate germline development in the pea aphid.

Literature cited

- Chang C-c, Lin GW, Cook CE, Horng SB, Lee HJ, Huang TY (2007) *Apvasa* marks germ-cell migration in the parthenogenetic pea aphid *Acyrtosiphon pisum* (Hemiptera: Aphidoidea). *Dev Genes Evol* 217: 275-287.
- Chang C-c, Huang TY, Shih CL, Lin GW, Chang TP, Chiu H, Chang WC (2008) Whole-mount identification of gene transcripts in aphids: Protocols and evaluation of probe accessibility. *Archives of Insect Biochem and Physiol* 68: 186-96.
- Chang C-c, Huang TY, Cook CE, Lin GW, Shih CL, Chen RP (2009) Developmental expression of *Apnanos* during oogenesis and embryogenesis in the parthenogenetic pea aphid *Acyrtosiphon pisum*. *Int J Dev Biol* 53: 169-176.

A new assembly of the pea aphid transcriptome incorporating EST data from four aphid organs

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Abstract

Pea aphid cDNA from four organs (bacteriocytes, embryos, fat body and gut) has been sequenced by 454 pyrosequencing. The data have been combined with published EST data to generate an updated database of aphid ESTs (available at www.aphidests.org). The 454 data also offer a semi-quantitative measure of transcript abundance, providing insight into the functional specialization of the different organs.

The power of combining genome annotation and systems level modeling

Metabolism Group: Sandy J. Macdonald^{1*}, John Ramsey², Georg Jander³, Atsushi Nakabachi⁴, Gavin H. Thomas¹, Alex C. C. Wilson⁵, Peter D. Ashton¹, Federica Calevro⁶, Hubert Charles⁶, Stefano Colella⁶, Gerard Febvay⁶ and Angela E. Douglas⁷

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Abstract

New insights into the impact of the *Buchnera* symbiosis on the complement and function of aphid genes are being gained by combining genome annotation by the Metabolism Group with reconstruction of the metabolic networks and systems level analysis of metabolite flux. We will illustrate the opportunities afforded by combining genomics and systems biology by considering (1) a previously unsuspected complementarity in the purine metabolism of the pea aphid and its symbiotic bacteria *Buchnera*, and (2) new data that clarify a long-standing paradox in our understanding of essential amino acid synthesis in the symbiosis.

Update on Ordering of Pea Aphid Scaffolds by HAPPY Mapping

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Abstract

HAPPY mapping is the analysis of approximately HAPloid DNA samples using the PolYmerase chain reaction. Intact nuclei isolated from adult pea aphids are immobilized in agarose strings, the DNA fragmented to a known size and dispensed in 0.5x genome equivalents into 96 wells of a mapping panel. The DNA is then randomly amplified by whole genome PCR, and the DNA fragment size and genomes per aliquot (gpa) tested. Once a mapping panel with the correct fragment range and gpa has been identified, STS markers chosen from the ends of sequence scaffolds can be rapidly typed onto the mapping panel by a series of multiplex and hemi-nested PCR's. The aim is to link the ends of scaffolds together to help assemble, orientate and position sequence scaffolds along the chromosomes, providing positional context for the contigs, and a resource of comparative mapping of related aphid species to investigate genomic synteny.

Here we give a progress report on the creation of a mapping panel and its validation using a set of markers designed against the 10 largest scaffolds from the June 2008 assembly.

The functional role of *A.pisum* sugar transporter 1 (ApST1) from the aphid gut.

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Abstract

The pea aphid genome sequence has been used to identify a complete set of facilitative sugar transporters. *A.pisum* sugar transporters (ApSTs) have been divided into two classes, based on an Interpro signature diagnostic for transporters of sugars and inositol (IPR003663). Of the 28 potential ‘true’ sugar transporters containing IPR003663, 8 genes have no corresponding ESTs, leaving 20 active genes. A similar analysis shows that *D. melanogaster* contains 17 ‘true’ sugar transporter genes, of which 14 have corresponding ESTs. Many of the identified *A.pisum* sugar transporters appear to have arisen from relatively recent gene duplication events, as shown by a cluster of 7 sugar transporter genes in a single 250kbp region of genomic DNA, and phylogenetic analysis of protein sequences. Duplication of sugar transporter genes leads to diversity, allowing functional adaptation to differences in requirements for the transport of sugars and other small molecules. As a result of specialised feeding habits in different insects, the complement of transporters differs between species. We are interested in sugar transformation and transport in the aphid gut and have begun a functional characterization of a subset of putative sugar transporters that are enriched in gut tissue. Transporters belonging to the major facilitator superfamily (MFS) are notoriously promiscuous, making it difficult to predict a specific transport function from only sequence data. Gut enriched putative ApST genes were validated as sugar transporters by functional complementation of a *Saccharomyces cerevisiae* strain EBY.VW4000, which has all of its hexose transporters deleted. We have previously characterised a uniporter (ApST3) which transports fructose>glucose. In contrast to ApST3, sugar transport by ApST1 is dependent on pH, and ApST1 is shown to act as a proton-coupled symporter for glucose. Both transporter genes have unusually high K_m values (ApST1 K_m for glucose is 100 mM), which may reflect a specific adaptation to utilization of plant phloem sap, which is very sugar rich and can be up to 1000 mM sucrose. ApST1 is strongly expressed throughout the midgut and we suggest exploits proton gradients created by V-type ATPase activity to allow glucose uptake against a concentration gradient. This mechanism contrasts with higher animals, where active glucose uptake from the gut lumen is mediated by a sodium-glucose symporter.

Using chemical mutagenesis for forward genetics on the pea aphid: utopia or reality?

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Abstract

Forward genetics has been used for many years to create mutations affecting the development of model organisms. Thanks to the emergence of modern genetics and genomics, it is now becoming easier to identify the genes disrupted by those mutations, although it remains the limiting step of this approach. One of the most widely used method of mutagenesis is based on feeding ethyl-methane sulfonate (EMS), an alkylating agent which induces modifications of the DNA sequence (from single-nucleotide changes to small deletions). Most mutations induced by EMS are recessive, which means that a phenotype becomes visible only when cells are homozygous for one mutation. Several generations are thus required to generate homozygous individuals for each mutation after mutagenesis (3 for an F3 screen). In addition, nucleotide changes induced by EMS occur randomly in the genome (in coding or non-coding regions alike), which implies that the frequency of significant changes is very low and that a large number of individuals have to be screened and handled to find a detectable phenotype. An EMS screen is thus a large-scale project, restricted mainly to model species, which are easy to rear, with small body size and a short generation time such as *Arabidopsis thaliana*, *Caenorhabditis elegans*, or *Drosophila melanogaster*.

Aphids develop several unique phenotypes linked to phenotypic plasticity. Through parthenogenesis reproduction, several alternative morphs from the same genotype can be produced such as winged or wingless, as well as sexual or asexual morphs. The knowledge of the molecular bases of this developmental trait which is not express in model eukaryotes is thus dependent on progresses that could be made in aphid post-genomics studies. With the availability of the pea aphid genome, as well as the very fast evolution of DNA sequencing for the next future, we want to check whether forward genetics can be applied to a non-model species (the pea aphid) with a long generation time (one generation per year, including the fundatrix effect) and the requirement to rear them on alive plants.

In this presentation we will first explain the ongoing protocol we are currently using to determine the conditions for an effective application of EMS treatment on the pea aphid. Second, we open the discussion on the feasibility of forward genetics using the pea aphid in the frame of the International Aphid Genomics Consortium community in order to coordinate an approach to generate and characterize pea aphid mutants on phenotypic plasticity.

Comparative genomic analyses of the odorant-binding proteins in the pea aphid *Acyrthosiphon pisum*

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Abstract

Chemoreception is an essential process for the survival of animals, allowing the recognition of volatile cues for the detection of food, predators and mates. Several gene families are implicated, including the chemoreceptor superfamily, the odorant-binding proteins (OBPs) and chemosensory proteins (CSPs). OBPs are a family of small water-soluble proteins that bind and solubilise hydrophobic odorants. They are abundant in the aqueous fluid surrounding olfactory receptor neurons and are involved in the first steps of olfactory signal transduction. These proteins are characterised by a specific domain that usually comprises six α -helices joined by three disulfide bonds and are classified into three OBP subfamilies: Classic, Plus-C and Atypical OBPs plus the CSPs. Using bioinformatic tools we have annotated genes encoding putative OBPs and CSPs in the pea aphid *Acyrthosiphon pisum*. In total we identified 13 Classic and two Plus-C OBPs and 13 CSPs. We also obtained, by experimental methods, the homologous OBP sequences from nine other aphid species. The comparative genomic analysis shows that paralogous OBP sequences are very divergent; nevertheless, orthologs are quite similar among aphids and the phylogenetic relationships reflect the divergence of the aphid species. In addition, we have found a few common orthologous groups between the pea aphid and its closest sequenced species, *Pediculus humanus*. Only two OBPs have clear orthologs across the insect phylogeny. Overall, our results support the ‘birth-and-death’ model as the major mechanism explaining aphid OBP sequence evolution, with purifying selection as the main evolutionary force.

DNA methylation in the pea aphid *Acyrtosiphon pisum*.

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Abstract

DNA methylation of cytosine residues is common in many eukaryotic genomes and is most commonly found at CpG sites. Recent evidence from the honeybee (*Apis mellifera*) and several other insect species has suggested a greater than hitherto suspected role for DNA methylation in insects. The sequencing of the *Acyrtosiphon pisum* genome has revealed the full complement of DNA methyltransferases and a number of genes with methylation at CpG sites within the coding sequence have been identified. This pattern of intragenic methylation and the apparent absence of intergenic methylation makes the pea aphid a good model organism for the study of methylation within the coding sequence of genes. Moreover, calculations of the CpG ratio of the coding sequences from the genome suggest that many genes could be methylated. In addition, DNA methylation has been observed in the machinery that controls microRNAs. In vertebrates, non-coding RNAs such as microRNAs can regulate and be regulated by DNA methylation. Recently it was discovered that the microRNA machinery in the pea aphid is duplicated making aphids unique within the Metazoa. Bisulphite sequencing has revealed that in some cases, DNA methylation is restricted to one of the duplicated copies. Preliminary evidence suggests that this methylation is positively correlated with expression of the gene. What still remains to be discovered is the overall pattern and functional significance of DNA methylation in insects and the interaction of DNA methylation with other transcriptional and post-transcriptional regulators of gene expression.



Proposal for the future of the International Aphid Genomics Consortium

Proposal for the future of the International Aphid Genomics Consortium

Prepared by Denis Tagu and Alex Wilson following discussions with IAGC members at Pea Aphid Genome Annotation Workshop I, July 14 & 15th 2008, Princeton, NJ, USA.

In Paris 2003, when Denis Tagu proposed the consortium be named "The International Aphid Genomics Consortium" (IAGC), he deliberately chose not to focus the name on a single aphid species nor the sequencing of a single genome. Publication of Acyr 1.0 together with our community's analysis of this initial assembly should be seen as the beginning of the IAGC's activities. We envision a strong future for the IAGC in building genomic and post-genomic resources for several aphid species.

There are three elements we believe are key to the community remaining cohesive and moving forward effectively:

- (1) A new IAGC White Paper. The new White Paper should identify the main objectives of the IAGC following publication of the pea aphid genome and be prepared and circulated by early December 2009.
- (2) Regular IAGC Workshops. Regular IAGC Workshops are needed to maintain our strong and active international community. The goal of the Workshops should be to strengthen the community via cooperation rather than competition. The aphid community is stronger united "against" other communities than we are alone as individual labs. Further, working as a community, will facilitate collaboration with other communities (e.g. locust, daphnia, white fly). Collaboration is important to get national and international funding. By collaborating we have the possibility to coordinate massive scientific programs, such as the sequencing of new genomes, full annotation, population genomics at a worldwide scale, complete transcriptomic and proteomic approaches under nearly exhaustive conditions, sharing of mutant RNAi screening and so on.
- (3) Appointment of an IAGC or Aphid Board.

How to proceed?

1. Workshops
 - a. Change the title of the workshops from "Pea Aphid Genome Annotation Workshop" to "International Aphid Genomics Consortium Workshop"
 - b. Define workshop frequency. Every 12 months, 18months or 2 years?
 - c. Ensure that each workshop has a special focus.
2. Formation of the IAGC Board (see proposal below)
3. Formation of IAGC/Aphid Board nomination committee.
4. Discussion of items targeted for discussion in the 2009 IAGC White Paper.

Proposal for the formation of an IAGC or Aphid Board

Purpose: Sequencing of the pea aphid genome has resulted in a significant expansion and strengthening of the aphid research community. The achievements of our community in the past 12 months necessitate the formation of an IAGC or Aphid Board whose mission and purpose is provide community leadership and facilitate progress in aphid biology through, (1) preparation and dissemination of an annual aphid white paper and (2) organization of regular IAGC Workshops.

Board Responsibilities

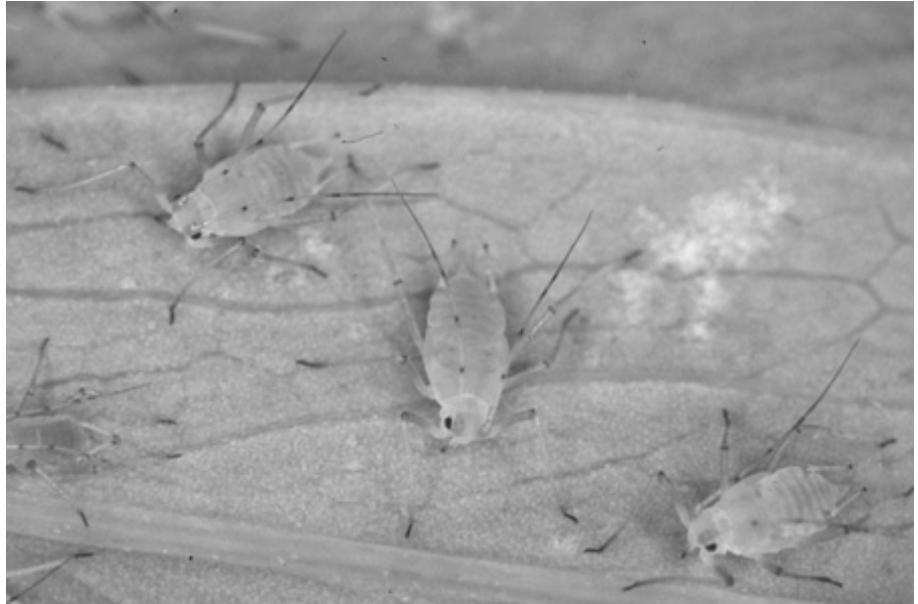
- To serve as advocates for the aphid research community and represent community interests for advising funding agencies by the preparation of an annual aphid white paper
- To facilitate a free and productive relationship between the research community and the administrators of AphidBase
- To insure successful regular IAGC Workshops. The board selects the venue and appoints the scientific organizer(s) of the workshop.

Structure: The Aphid Board is an elected group of working scientists, one from Europe, one from America and one from Asia/Oceania, who use aphids as their primary model organism. Service on the Board is for a period of two years; with the exception that one of the three founding Board members serves for an initial period of three years so as to ensure continuity and long-term memory of the Board.

The Board meets regularly in conjunction with the IAGC Workshop. Additional business is conducted by email, the Aphid Collaboration Wiki and, if necessary, by telephone conferences.

The Board's discussion of community issues benefit from input from the entire community. It is the responsibility of Board members to canvass aphid researchers residing in their regions so input can be obtained on major issues of concern.

Elections: The "past-President" or in the case of the first election, David Stern, will be responsible for organizing the election of the three Board members. A nomination committee will be formed at Pea Aphid Genome Annotation Workshop II, June 4-5th 2009, Barcelona, to name two delegates for each position to be elected. Delegates living in the different regions are chosen to ensure diversity and broad representation on the Board. During the first election, everyone who is an author on the pea aphid genome paper is eligible to vote. The first elections will be held in July 2009. The founding Board members will establish future issues of governance of the Board.



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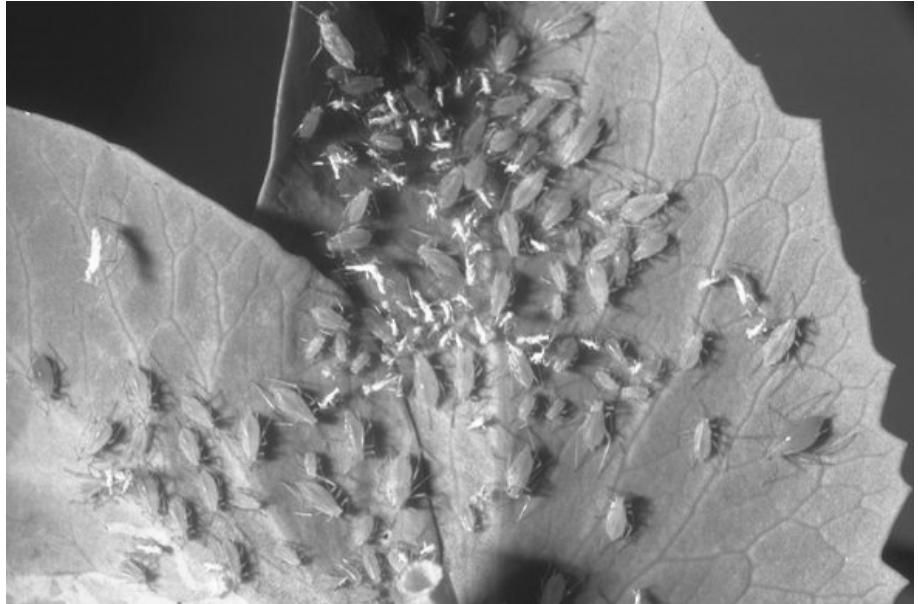
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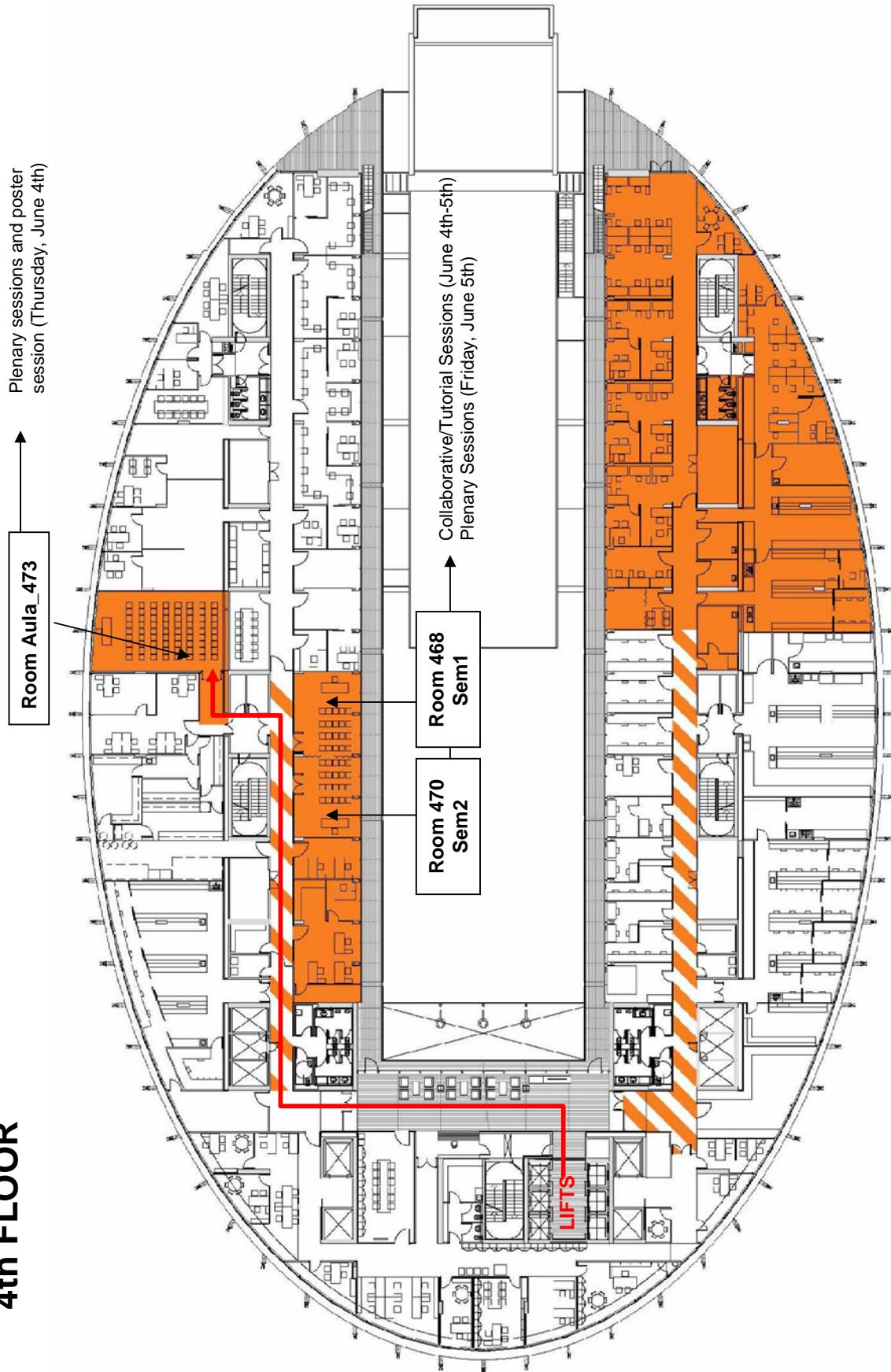
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