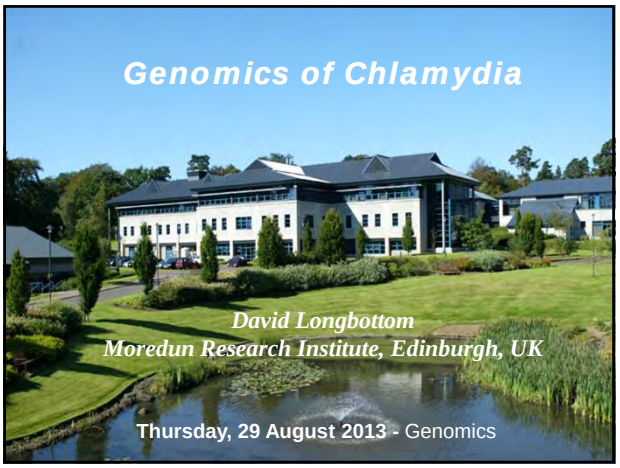


Chlamydial Taxonomy

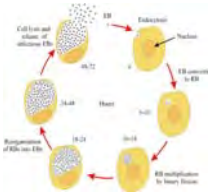
Period	Phylum	Class	Order	Family	Genus	Species	References
Pre-1998	Chlamydiae	Chlamydiae	Chlamydiales	Chlamydiaceae	Chlamydia	C. trachomatis C. psittaci C. pneumoniae C. pecorum	Grayston et al., 1989, IJSB 39,88. Fukushi & Hirai, 1989, IJSB 42, 306. Longbottom & Coulter, 2003, J Comp Path 128, 217.
Post 1998	Chlamydiae	Chlamydiae	Chlamydiales	Chlamydiaceae [Parachlamydiaceae; Simkaniaceae; Waddliaceae]	Chlamydia Chlamydophila	C. trachomatis C. muridarum C. suis C. pneumoniae C. pecorum C. felis C. caviae C. psittaci C. abortus	Everett et al., 1999, IJSB 49, 415. Schachter et al., 1999, IJSB 51, 249. Everett & Andersen, 2001, IJSEM 51, 2181.
Current	Chlamydiae	Chlamydiia	Chlamydiales	Chlamydiaceae [Criblamydiaceae; Parachlamydiaceae; Rhabdochlamydiaceae; Simkaniaceae; Waddliaceae; Candidatus Parilichlamydiaceae]	Chlamydia	C. trachomatis C. muridarum C. suis C. pneumoniae C. pecorum C. felis C. caviae C. psittaci C. abortus	Kuo et al., 2011. In: Bergey's Manual of Systematic Bacterology, 2nd Edn., Vol 4. pp. 846-865.

Family Chlamydiaceae

Chlamydia sp.	Typical Host	Infection/Disease
C. trachomatis	Humans	STI, PID, salpingitis, trachoma
C. pneumoniae	Humans, Koala, Horse	Pneumonia, bronchitis
C. muridarum	Mice, hamsters	Respiratory, genital, (Model System)
C. caviae	Guinea pig	Ocular, genital, (Model System)
C. felis	Cats	Conjunctivitis
C. psittaci	Birds, poultry	Respiratory
C. abortus	Ruminants, swine	Abortion
C. suis	Swine	Enteric, respiratory, reproductive
C. pecorum	Ruminants, swine	Enteritis, conjunctivitis, metritis, mastitis, polyarthritis, pneumonia, encephalomyelitis

Chlamydiaceae: Genome sequencing

- Infect eukaryotic host cell for replication
  - intracellular life style
- Share a biphasic developmental cycle
- Major differences in host range, tissue tropism and disease pathogenesis
- Comparison of genes involved either directly or indirectly in the interactions with the host cell will shed light on the evolution of this intracellular life style and adaptation in different eukaryotic hosts.



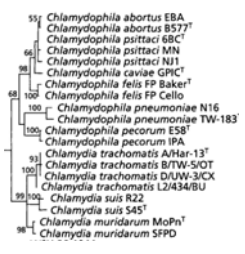
Moredun

"First Wave" Chlamydial Genome Sequencing

- *Chlamydia trachomatis* serovar D strain D/UW-3/CX (University of California, Berkley, USA)
  - Stephens et al., 1998. Science 282, 754.
- *Chlamydophila pneumoniae* strain CWL029 (Stanford University, California, USA)
  - Kalman et al., 1999. Nat Genet 21, 385.
- *Chlamydophila pneumoniae* strain AR39 (The Institute for Genomic Research, Maryland, USA)
- *Chlamydia muridarum* MoPn strain (TIGR, Maryland, USA)
  - Read et al., 2000. Nucleic Acids Res 15, 1397.
- *Chlamydophila pneumoniae* strain J138 (Yamaguchi University, Japan)
  - Shirai et al., 2000. Nucleic Acids Res 28, 2311.
- *Chlamydophila caviae* GPIC strain (TIGR, USA)
  - Read et al., 2003. Nucleic Acids Res 31, 2134.
- *Chlamydophila abortus* S26/3 strain (Wellcome Trust Sanger Institute, UK)
  - Thomson et al., 2005. Genome Res 15, 629.
- *Chlamydophila felis* strain Fe/C-56 (Yamaguchi University, Japan)
  - Azuma et al., 2006. DNA Res 13, 15.

Moredun

Chlamydial Genome Sequencing Projects



Species	Chromosome	Contigs	Short Read Archive	No data
C. abortus	1	1		67
C. psittaci	18	27	12	28
C. caviae	1			
C. felis	1			
C. pneumoniae	5			1
C. pecorum	1			3
C. trachomatis	75			24
C. suis				1
C. muridarum	2	2		1
TOTAL	104	30	12	125

Chlamydiae Genomes Re-annotation Initiative - http://www.chlamydiaeDB.org

General features of chlamydial genomes

	C. trachomatis (serovar D)	C. muridarum (Nigg)	C. pneumoniae (AR39)	C. caviae (GPIC)	C. abortus (S26/3)	C. felis (Fe/C-56)	C. pecorum (E58)	C. psittaci (6BC)
Genome (bp)	1,042,519	1,072,950	1,229,858	1,173,390	1,144,377	1,166,239	1,106,197	1,171,660
% GC of genome	41.3	40.3	40.6	39.2	39.9	40.0	41.1	39.1
% coding	90.1	90.0	89.0	89.4	88.2	91.2	91.9	89.4
Predicted CDS	894	921	1130	1009	961	1005	1073	967
tRNAs	37	37	38	38	38	38	38	38
rRNA operon	2	2	1	1	1	1	1	1
No. Pmp genes	9	9	21	18	18	20	15	21
dsDNA plasmid	7,493	7,501	-	7,966	-	7,552	-	7,553

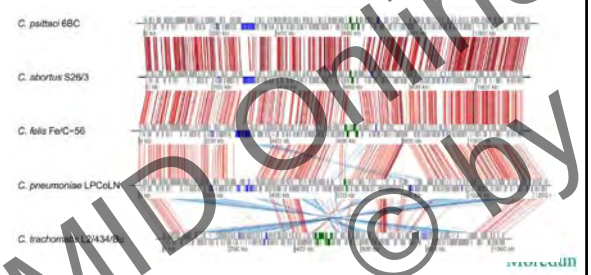
Genome sequencing

- Evidence for an obligate intracellular lifestyle already known
  - Trend towards a reduced genome size
  - Sequence divergence in proteins that mediate interactions with the host environment
    - Outer membrane proteins
- No evidence of recent genome reduction
  - such as low gene density or an unusual number of degraded open reading frames (ORFs) and pseudogenes

Moredun

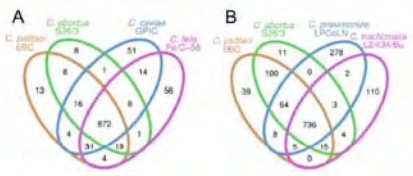
Comparative genomic analysis

- High level of conservation in both sequence and overall gene content



Comparative genomic analysis

- Common (core) gene set likely reflects the lifestyle of an intracellular organism that has achieved an optimal balance with its host
- Unique (species-specific) genes likely involved in giving rise to specific phenotypes, host specificity and ecological/biological niches



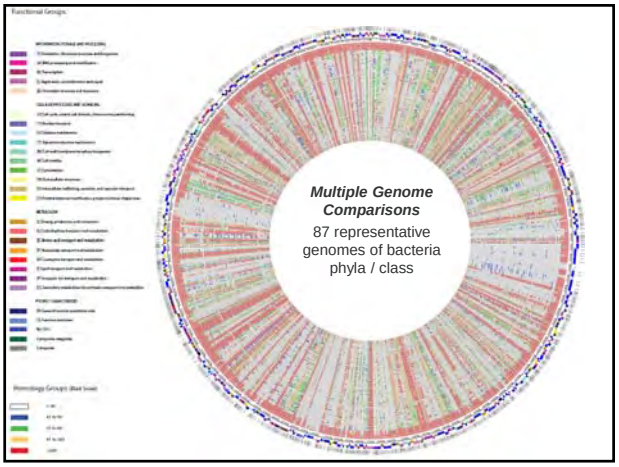
From Voigt et al., 2012. PLOS One 7, e35097

Moredun

Chlamydiaceae: common features

- Approx 75% of predicted proteins were found to be conserved
- May represent minimal gene content required for intracellular survival and development cycle
  - Essential metabolic functions
  - Host interaction
- Orthologs to genes in other bacteria identified accounting for the minimal requirements for DNA replication, repair, transcription and translation

Moredun



Chlamydiaceae: common features

- Approx 75% of predicted proteins were found to be conserved
- May represent minimal gene content required for intracellular survival and development cycle
  - Essential metabolic functions
  - Host interaction
- Orthologs to genes in other bacteria identified accounting for the minimal requirements for DNA replication, repair, transcription and translation
- Extensive repertoire of genes for DNA repair and recombination systems
  - Evidence of genome-wide recombination in *C. trachomatis* (Harris et al., 2012. Nat Genet 44, 413)
  - Detailed phylogeny on 52 representative strains from trachoma and LGV biovars
  - How this genetic exchange manifests into ocular, urogenital and lymphogranuloma venereum strains

Moredun

Highlights: Core metabolism

- Pre-genomics chlamydial dogma stated that chlamydiae were "energy parasites"
  - Evidence of an extensive energy-generating capability
    - An almost complete tricarboxylic acid cycle (aspartate shunt)
    - Glycolytic pathway
    - Pentose phosphate pathway
- Fully functional electron transport chain
  - Re-oxidation of reduced co-factors produced by TCA cycle
  - Encode V-ATPase enzyme complex genes found in eukaryotes and plastids rather than F-type ATPases seen in bacteria
- Glycogen accumulation/storage (*C. trachomatis*)
  - Complete glycogen synthesis and degradation system
  - Central role for glucose as a primary carbon source for some developmental stages in chlamydial biology

Moredun

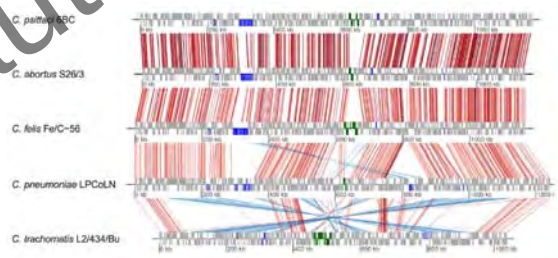
Highlights: Central metabolism

- Absence of biosynthetic pathways for key amino acids and nucleotides, making them dependent on host for amino acid intermediates
  - Amino acid and peptide transporters
- Presence of nearly all genes required for peptidoglycan biosynthesis
  - Muramic acid not previously detected or detected in small amounts
  - Might explain why chlamydiae only partially sensitivity to penicillin
  - Chlamydiae synthesise small amounts of peptidoglycan or peptidoglycan-like component
  - Cysteine-rich proteins (OmcA, OmcB)
- Absence of gene encoding the key cell division protein FtsZ, which is present in almost all other eukaryotes and prokaryotes
  - Maybe a reflection of the small amount of peptidoglycan in chlamydiae

Moredun

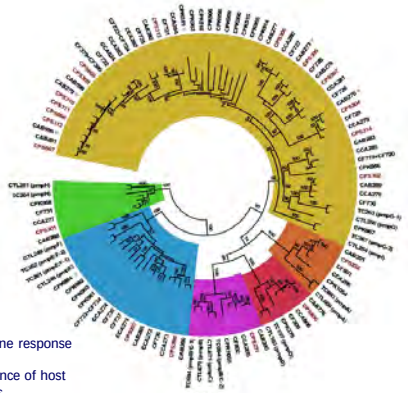
Comparative genomic analysis

- Evidence of genomic rearrangements with reciprocal recombination events in hypervariable regions near the replication terminus (plasticity zone) and near the major Pmp cluster



Chlamydial pmp diversity

Type V Secretion System

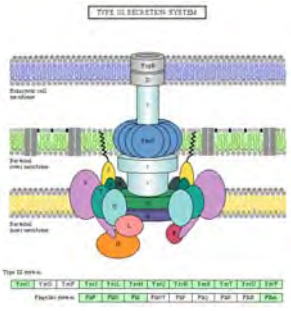


- Trigger inflammatory immune response
- Bacterial invasion
- Antigenic diversity – avoidance of host adaptive immune responses

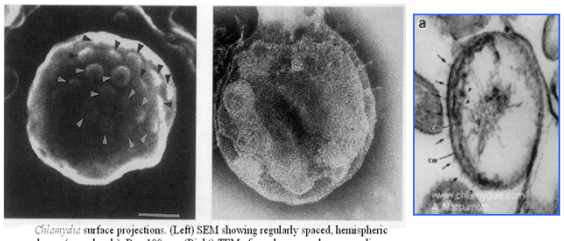
From Voigt et al., 2012. PLOS One 7, e35097

Type III Secretion System

- Chlamydiae contain complement of genes encoding structural, regulatory and chaperone components of a type III secretion system
- Clustered in 4 chromosomal regions
- Transport of T3 effector into cell cytoplasm or targeted to inclusion membrane
- Virulence determinants for Gram-negative bacteria interfering with host cell signal transduction and other cellular processes (Hueck, 1998. Microbiol Mol Biol Rev 62, 379)



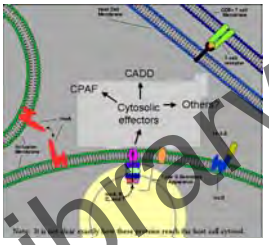
First clues of a T3SS



Chlamydia surface projections. (Left) SEM showing regularly spaced, hemispheric domes (arrowheads). Bar, 100 nm. (Right) TEM of envelope complexes revealing spike-like projections which are believed to extend through the inclusion membrane (Courtesy of A. Matsumoto; from *Chlamydia*, Intracellular Biology, Pathogenesis, and Immunity, R. S. Stephens (Ed.)).

T3SS Effector Proteins

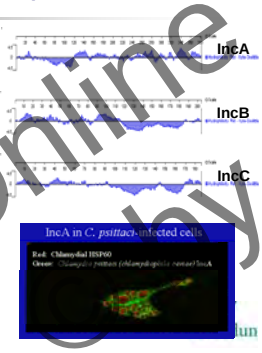
- **Cap1** - CD8⁺ T cell antigen class I accessible protein–1 (Balsara et al., 2006. J Infect Dis 193, 1459)
- **CopN** – sequesters tubilin & prevents microtubule formation (Fields & Hackstadt, 2000. Mol Microbiol 38, 1048; Archuleta et al., 2011. J Biol Chem 286, 33998)
- **Tarp** (Translocated actin-recruiting protein) – actin recruitment to sites of chlamydial attachment (Clifton et al., 2004. PNAS 101, 10166; Lane et al., PLOS Pathogens 2008 e1000014)
- **CPAF** (Chlamydia proteosome-like activity factor) **(T2SS)** – secreted protease that cleaves host transcription factors essential for MHC class I & II antigen presentation (Shaw et al., 2002. Cell Microbiol 4, 411; Chen et al., 2012. PLOS Pathogens 8, e1002842)
- **CADD** (Chlamydia protein associating with death domains) – binds DD-containing TNF family receptors (Fas) – prevents apoptosis by recruiting Fas to inclusion - induces apoptosis in mammalian cell lines when expressed by transient gene transfection (Stenner-Liewen et al., 2002. J Biol Chem 277, 9633)



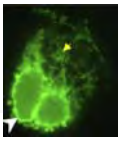
- **Inc** proteins – high diversity

Inclusion membrane proteins

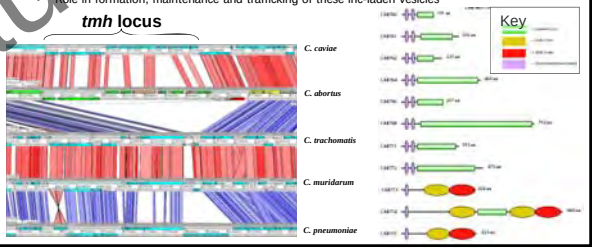
- Little or no primary sequence similarity
- Possess unique paired hydrophobic transmembrane domains
- Inc A to G
- Some (A to C) experimentally confirmed as T3S effectors
- Upwards of 50 additional putative incs identified
- IncA – required for vesicle fusion or septation of inclusion membrane during cell division (Hackstadt et al., 1999. Cell Microbiol 1, 119)
- IncB – modulates host immune responses and may be involved in inclusion development and prevention of early lysosomal fusion (Gupta et al., 2009. Reprod Biol Endocrinol 7, 38)
- Transmembrane Head (TMH) protein genes



Transmembrane head proteins

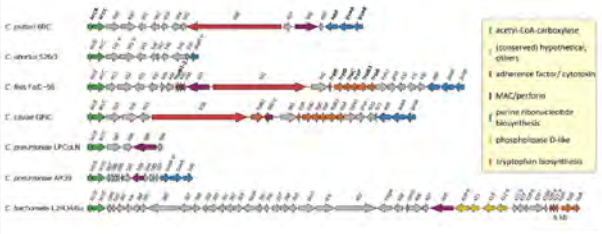


- Single TM domains + domains of unknown function
- Paired N-terminal hydrophobic TM domains (IncA) + α-helical coiled-coil domains
  - Inc766 – oligomers, extra-inclusion vesicles & loops (Vretou et al., 2008. Microb Pathog 45, 265)
  - Role in formation, maintenance and trafficking of these inc-laden vesicles



Plasticity Zone / Replication Termination Region

- Region most variable in gene content, size and organisation
- In keeping with rapid evolution of a set of putative virulence factors
- Virulence factors linked to host-pathogen interactions/pathogenesis & niche adaptation



Plasticity Zone / Replication Termination Region

- **Cytotoxin genes** (1x: CCA, CFE, CPS; 2x: CPE; 3x: CMU; ~~CTR~~)
  - Similar to EHEC adherence factor 1 (Efa1), lymphocyte inhibitory factor A (LifA) and clostridial large cytotoxins
  - May play a role in invasion-related reorganisation and remodelling of the actin cytoskeleton
  - Inhibit host cell signalling through glycosylation of small GTP-binding proteins (Eichel-Streiber et al., 1996. Trends Microbiol 4, 375)
  - Inhibit lymphocyte activation (Klapproth et al., 2000. Infect Immun 68, 2148)
  - Inhibit IFN-γ production by host cell enabling switch from persistent infection to acute disease
- **MAC/perforin** (CPS, CFE, CPN Koala, CTR, ~~CCA~~, ~~CPN~~)
  - Eukaryotic proteins function as membrane perforation proteins, playing important role in animal immune response to bacterial infections
  - In Chlamydia may be involved in evading host immune response through structural mimicry (Stebbins & Galán, 2001. Nature 412, 701)
- **Phospholipase D** [HKD superfamily] (4-5 genes: CTR, CPE)
  - Function unknown , but may be associated with inclusion formation (Nelson et al., 2006. Infect Immun 74, 73) or invasion of host cells

Plasticity Zone / Replication Termination Region

- **Tryptophan biosynthesis**
  - IFN-γ mediated breakdown of Trp by indoleamine-2,3-dioxygenase (IDO) – important in host defence against chlamydiae
  - Trp biosynthesis important in resistance against IFN-γ – role in persistent, subclinical infections?
  - Differences important for host tropism of individual chlamydial species

Species	Trp biosynthesis
C. trachomatis	*trpABCR*
C. muridarum	-
C. pneumoniae	*tph*
C. pecorum	*trpABFCDR kynU prsA* (not in PZ)
C. psittaci	-
C. abortus	*tph*
C. felis	*trpABFCDR kynU prsA*
C. caviae	*trpABFCDR kynU prsA tph*

Chlamydial genomics

- Many genes conserved across all genomes are part of unique metabolic repertoire
- Others like pmps are found in all genomes. Although deletions, expansions and recombination may give individual strains a unique complement of genes that confer specific phenotypes
- Biotype/Species-specific genes such as large chlamydial toxin and tryptophan biosynthesis genes may be key contributors to host/niche specificity and/or have a role in chlamydial persistence
- These genomic differences may account for the observed variations in virulence, pathogenicity and host specificity
- Definitive function for gene products?
  - Genetic manipulation of Chlamydia – stable transformation system??
  - *"Development of a Transformation System for Chlamydia trachomatis: Restoration of Glycogen Biosynthesis by Acquisition of a Plasmid Shuttle Vector."* Wang et al., 2012. PLoS Pathog 7, e1002258

Moredun

Thank you for your attention