

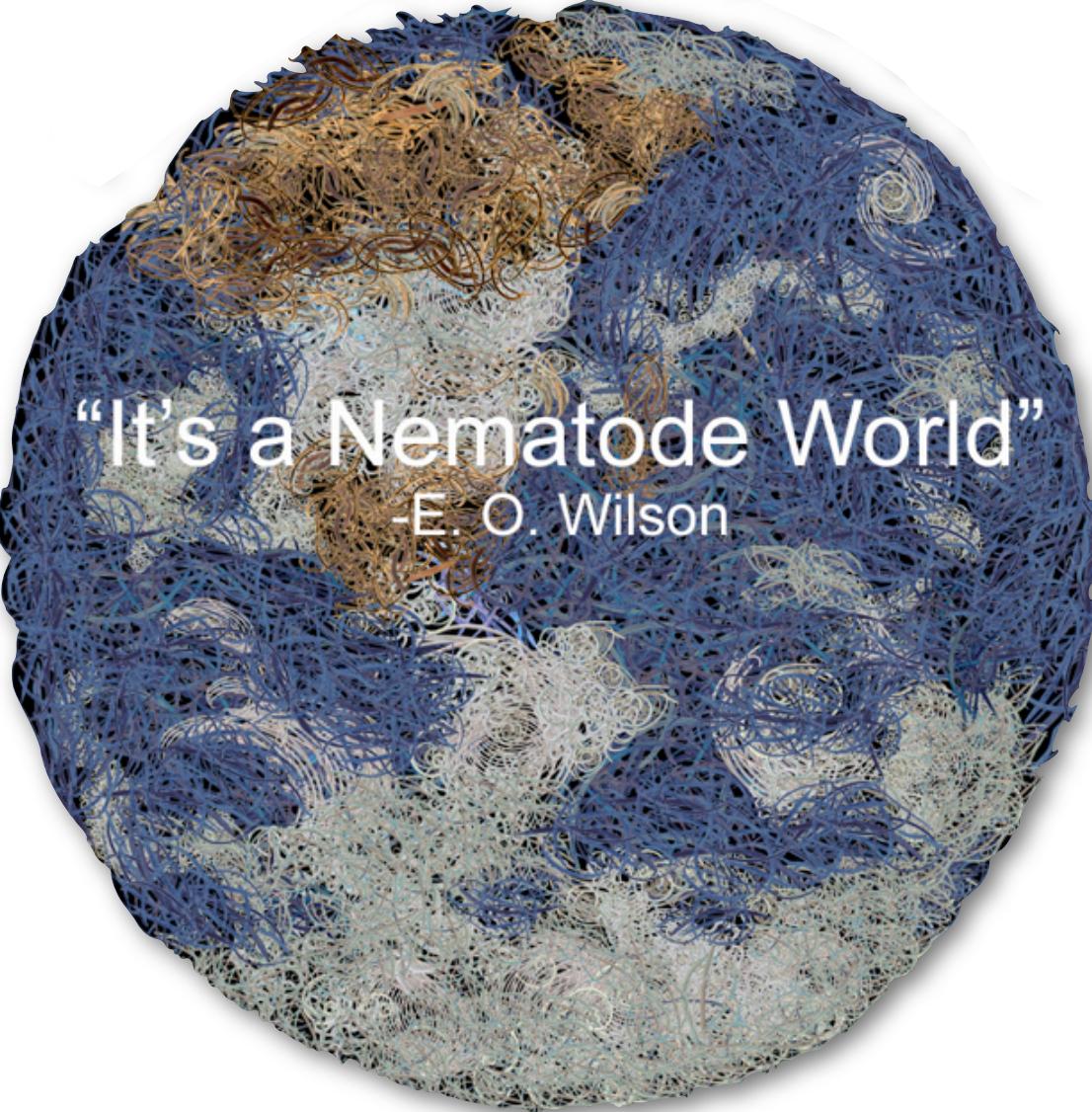
Genomics: Nematodes

Alan L. Scott

*W. Harry Feinstone Department of
Molecular Microbiology & Immunology*

*Johns Hopkins University
Bloomberg School of Public Health*





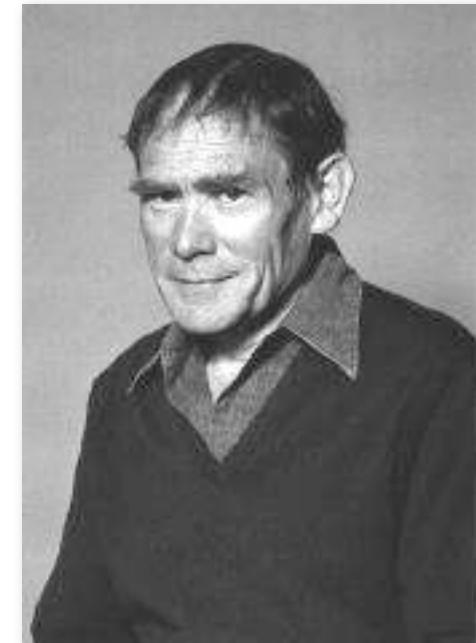
“It’s a Nematode World”
-E. O. Wilson



Excerpts from Proposal to the Medical Research Council, October, 1963

“Thus we want a multicellular organism which has a short life cycle, can be easily cultivated, and is small enough to be handled in large numbers, like a micro-organism. It should have relatively few cells, so that exhaustive studies of lineage and patterns can be made, and should be amenable to genetic analysis.”

“We think we have a good candidate in the form of a small nematode worm, *Caenorhabditis briggsae*, ...”

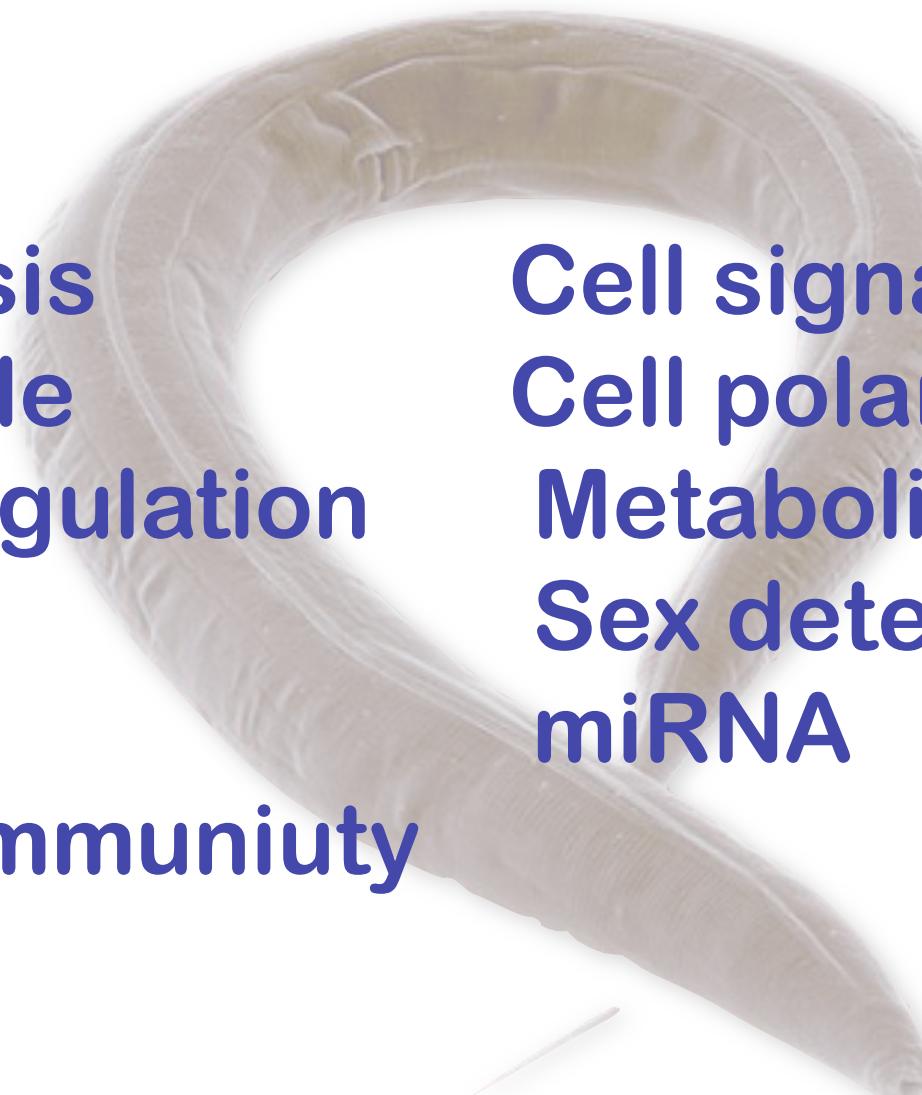


Sidney Brenner



C. elegans: model system for human disease

**Apoptosis
Cell cycle
Gene regulation
Ageing
RNAi
innate immunity**

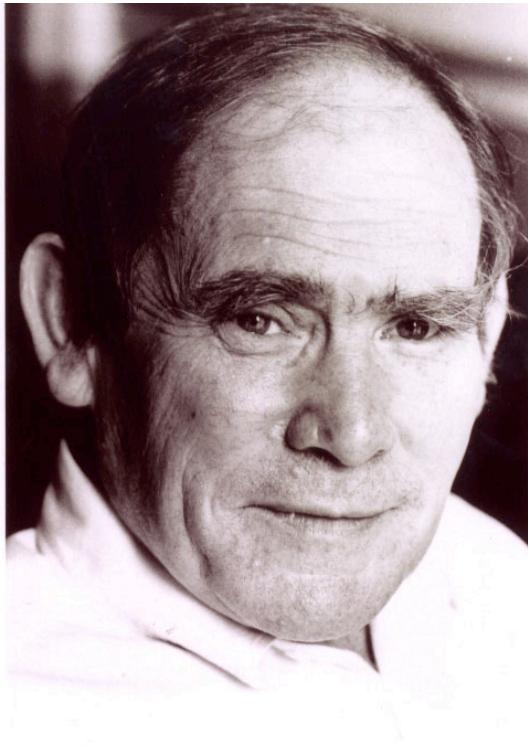


**Cell signaling
Cell polarity
Metabolism
Sex determination
miRNA**



Sidney Brenner

Sydney Brenner



F1 generation

Phil Anderson	Martin Chalfie
Padmanabhan Babu	David Hirsh
David Baillie	David Miller
Thomas Blumenthal	Judith Kimble
Bob Horvitz	Robert Waterston
John Sulston	John White
Samuel Ward	Donald L Riddle
Shahid Siddiqui	Johji Miwa
Henry Epstein	Laurent Segalat
Peter Candido	Edward Hedgecock
Ralf Schnabel	Bob Edgar
Robert K Herman	Hiroaki Kagawa
Anthony Otsuka	Barbara Meyer
Jonathan Hodgkin	William Sharrock
Cynthia Kenyon	Andrew Fire
Dick Russell	Ichi Maruyama

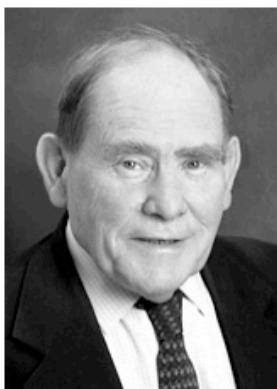


Sidney Brenner



The Nobel Prize in Physiology or Medicine 2002

"for their discoveries concerning 'genetic regulation of organ development and programmed cell death'"



Sydney Brenner

⌚ 1/3 of the prize

United Kingdom

The Molecular Sciences Institute
Berkeley, CA, USA

b. 1927



H. Robert Horvitz

⌚ 1/3 of the prize

USA

Massachusetts Institute of Technology (MIT)
Cambridge, MA, USA

b. 1947



John E. Sulston

⌚ 1/3 of the prize

United Kingdom

The Wellcome Trust Sanger Institute
Cambridge, United Kingdom

b. 1942



The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"



Photo: L. Cicero/Stanford

Andrew Z. Fire

⌚ 1/2 of the prize

USA

Stanford University
School of Medicine
Stanford, CA, USA

b. 1959



Photo: R. Carlin/UMMAS

Craig C. Mello

⌚ 1/2 of the prize

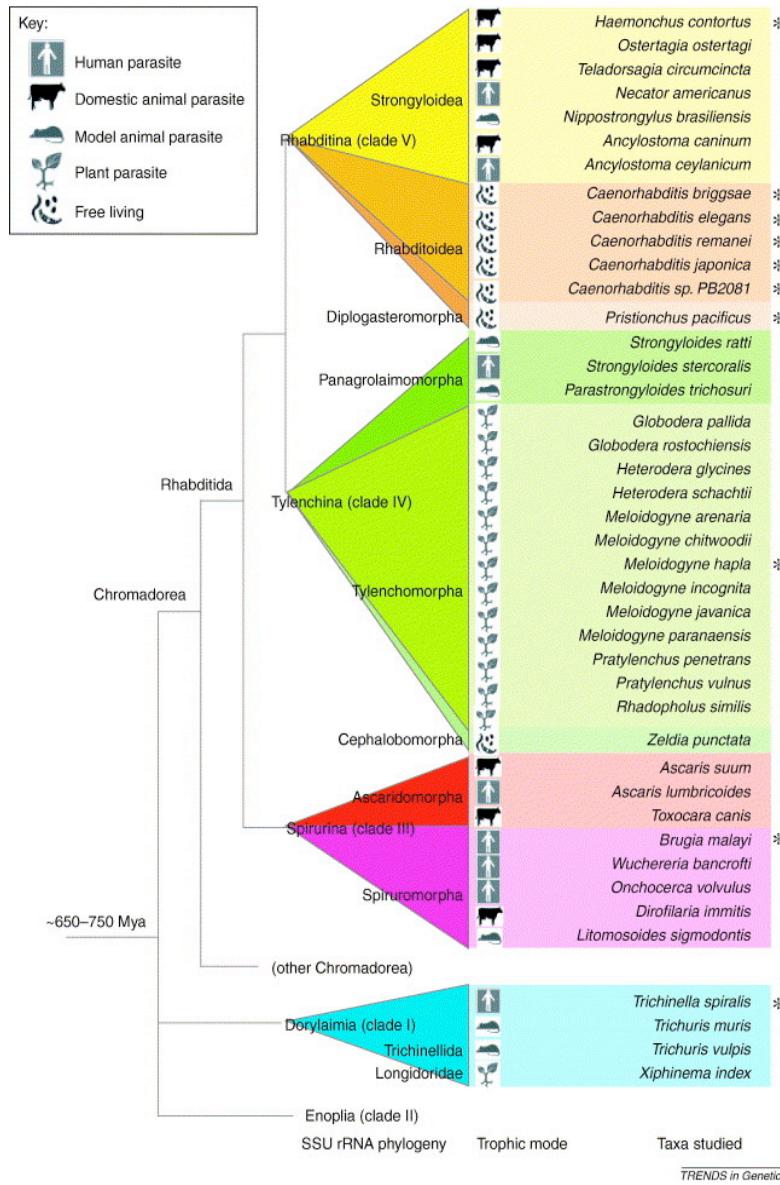
USA

University of Massachusetts Medical School
Worcester, MA, USA

b. 1960



Nematodes - speciose



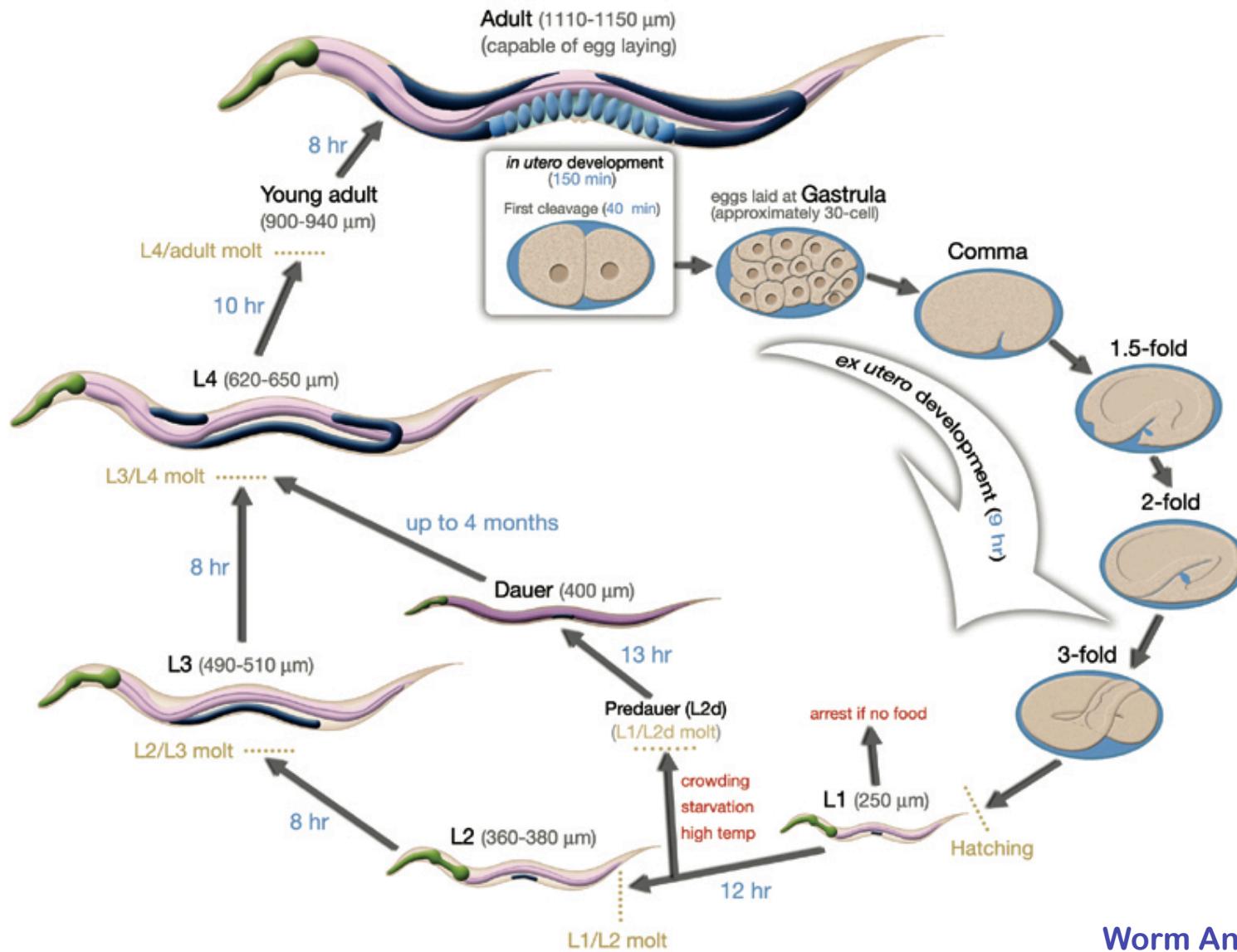
~ 10^6 nematode species

free-living
↓
phoresy
↓
necromony
↓
parasitism





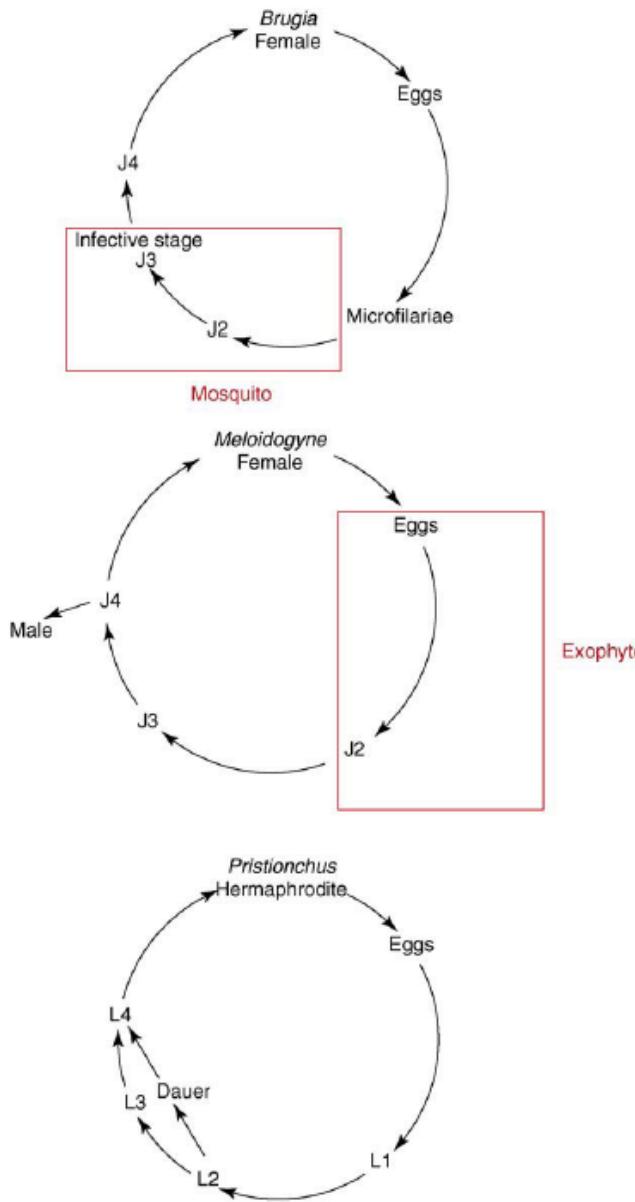
C. elegans life cycle



Worm Anatomy



life cycle options



- **parasitic (animal)**
- **diecious**
- **multiple hosts**
- **no free-living stage**
- **developmental arrest at L1 and L3**

- **parasitic (plants)**
- **mitotic parthenogenesis**
- **exophytic stage**

- **free-living**
- **hermaphroditic**
- **dauer formation**

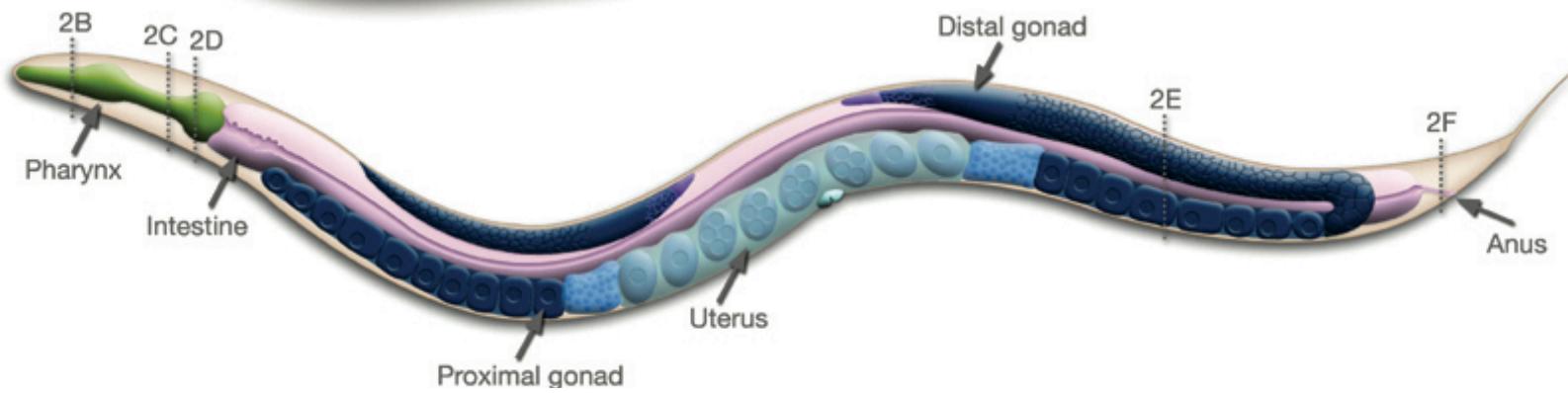


Caenorhabditis elegans

A.



B.



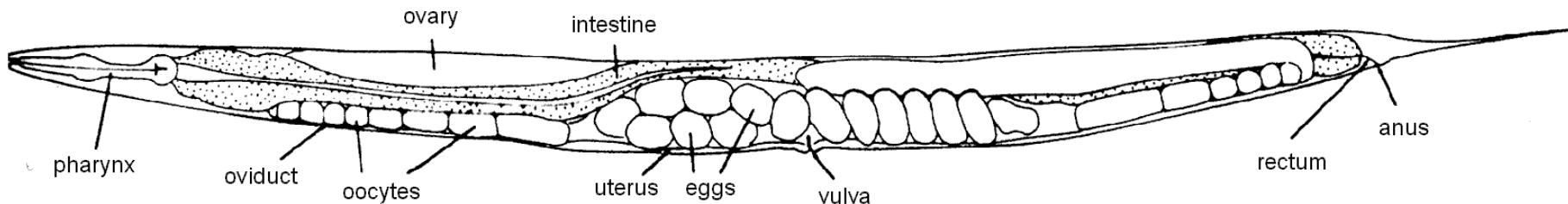
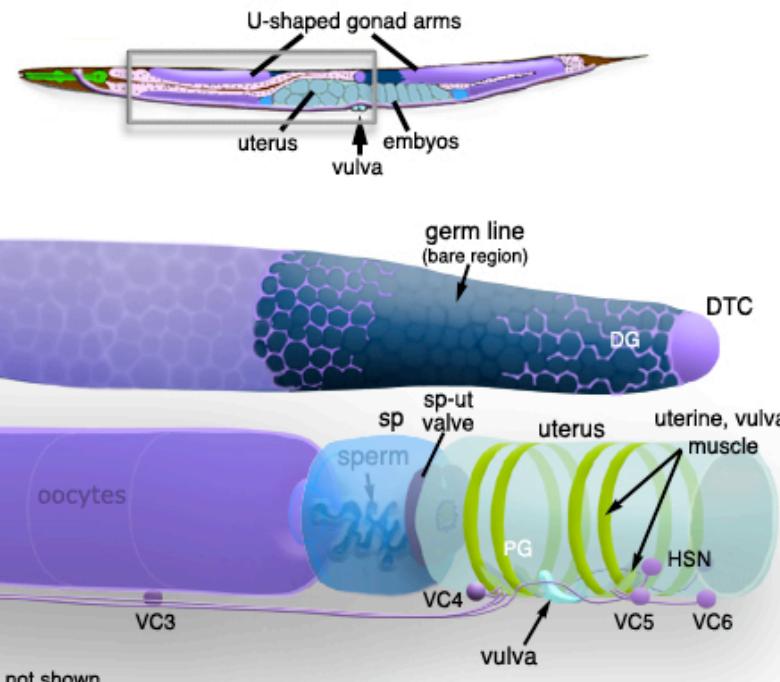
Worm Anatomy



C. elegans reproductive system

A database of behavioral and structural anatomy

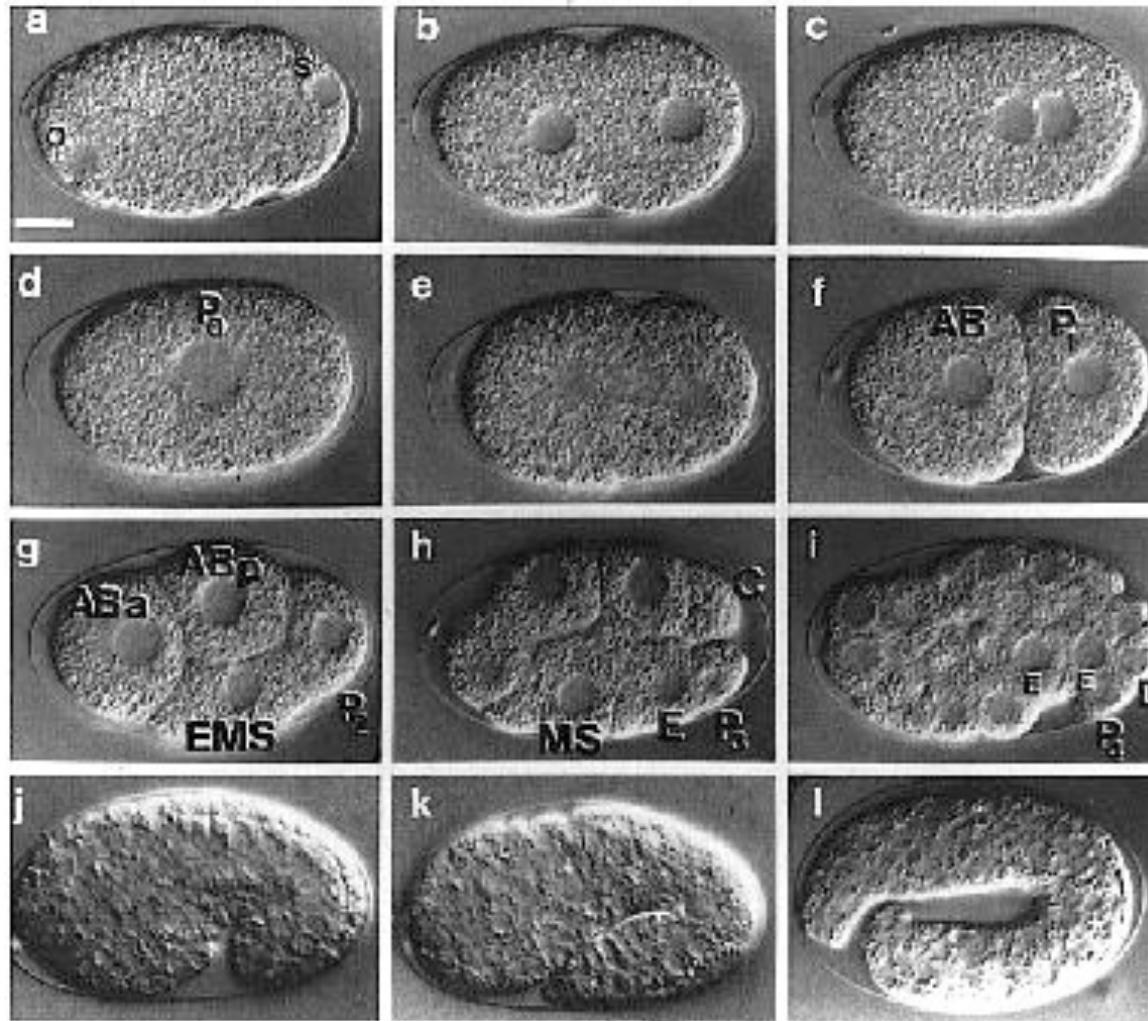
WORMATLAS
Caenorhabditis elegans





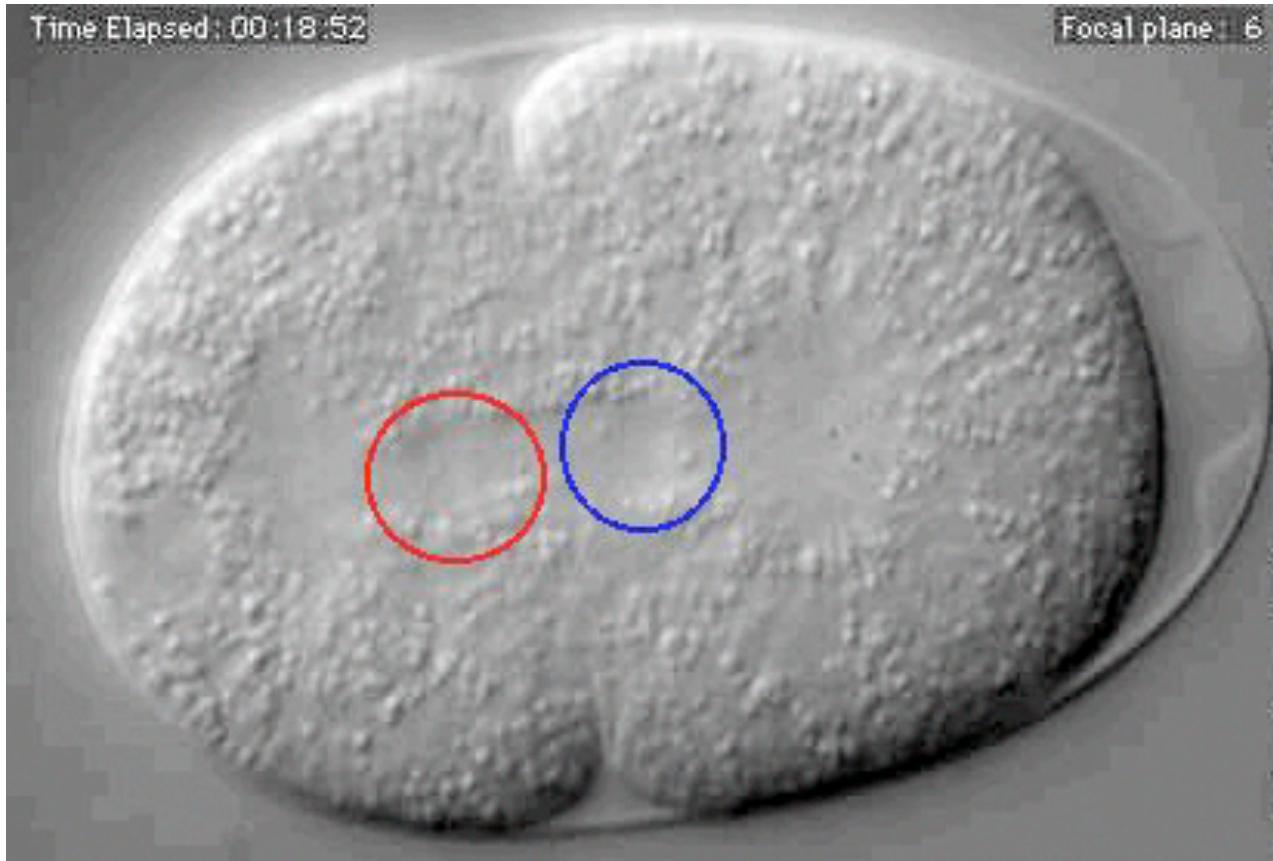
C. elegans embryonic development

Cell lineage



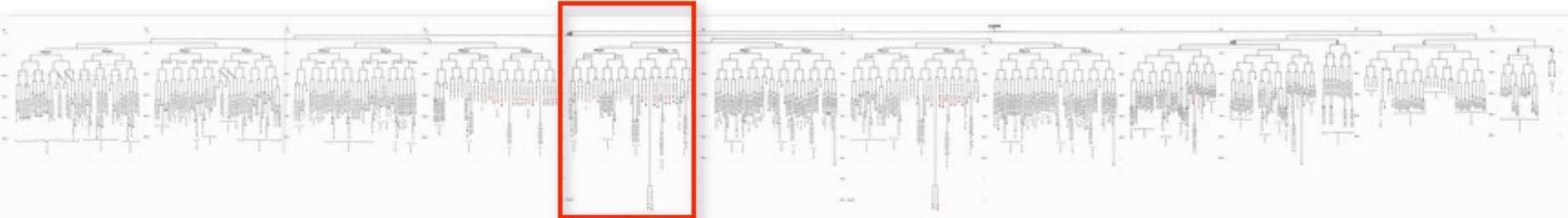


C. elegans the movie

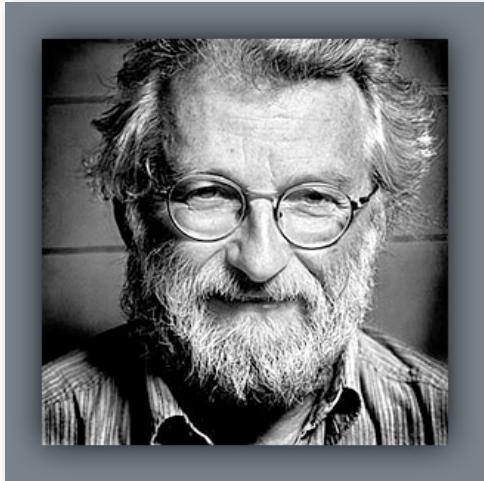


<http://embryology.med.unsw.edu.au/Science/ANAT2341lab02.htm>

<http://www.wormclassroom.org/modules/celllineage/CACE/CEE.html>



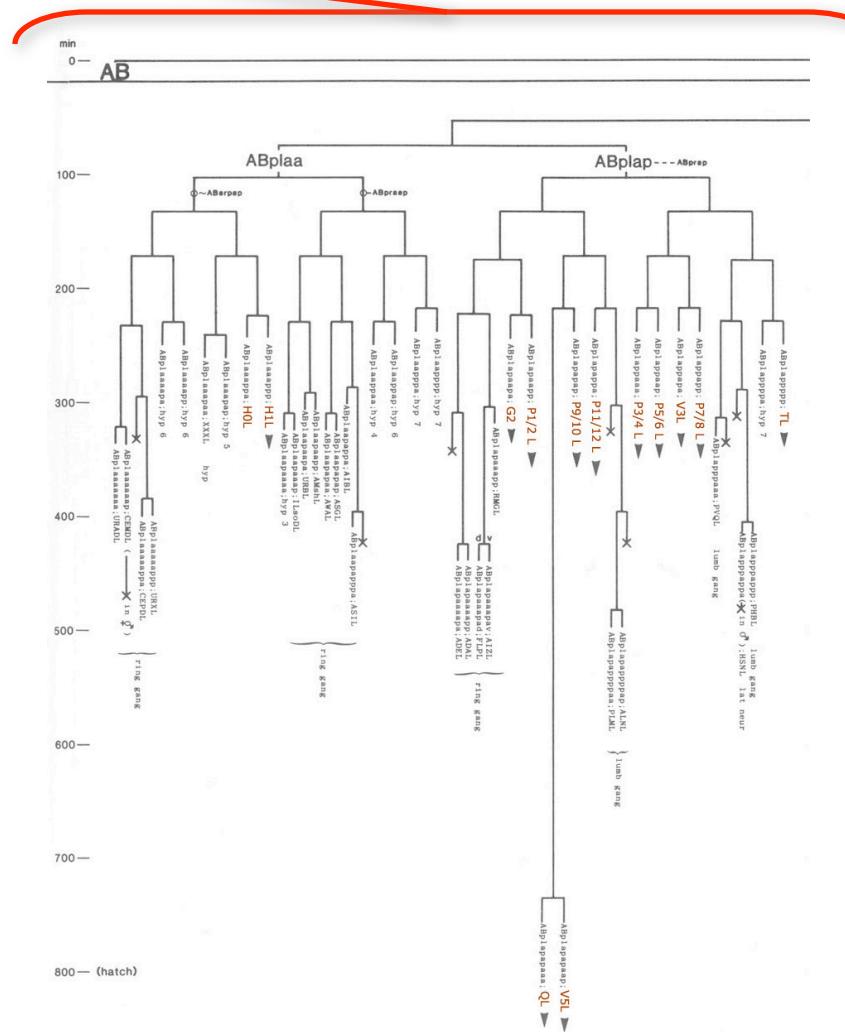
Cell lineage



Dr. (Sir) John Sulston spent one and a half years (in the dark) to complete the tracking of the 959 somatic cells of the adult *C. elegans* cell lineage.

He hand drew worm development in real time without the aid of video imaging.

Published in lineage in 1983.





First multicellular organism to be sequenced



1998

SPECIAL SECTION

C. ELEGANS: SEQUENCE TO BIOLOGY

Genome Sequence of the Nematode *C. elegans*: A Platform for Investigating Biology

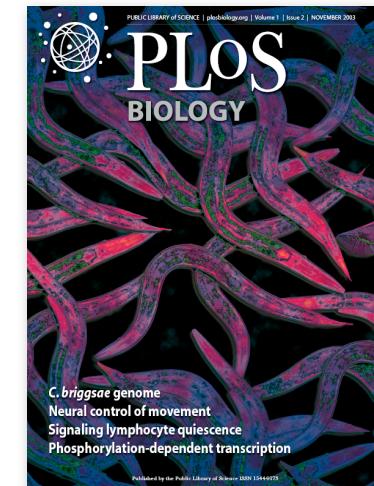
The *C. elegans* Sequencing Consortium*

The Genome Sequence of *Caenorhabditis briggsae*: A Platform for Comparative Genomics

PLOS BIOLOGY

2003. Vol 1:166

Lincoln D. Stein,^{1*} Zhirong Bao,^{2,9} Darin Blasius,³ Thomas Blumenthal,⁴ Michael R. Brent,⁵ Nansheng Chen,¹ Asif Chinwalla,³ Laura Clarke,⁶ Chris Cleo,⁶ Avril Coglan,⁷ Alan Coulson,^{6,13} Peter D'Eustachio,^{1,8} David H. A. Fitch,¹⁴ Lucinda A. Fulton,³ Robert E. Fulton,³ Sam Griffiths-Jones,⁶ Todd W. Harris,¹ LaDeana W. Hillier,^{3,9} Ravi Kamath,⁶ Patricia E. Kuwabara,⁶ Elaine R. Mardis,³ Marco A. Marra,^{3,10} Tracie L. Miner,³ Patrick Minx,³ James C. Mullikin,^{6,11} Robert W. Plumb,⁶ Jane Rogers,⁶ Jacqueline E. Schein,^{3,10} Marc Sohrmann,⁶ John Spieth,³ Jason E. Stajich,¹² Chaochun Wei,⁵ David Willey,⁶ Richard K. Wilson,³ Richard Durbin,⁶ Robert H. Waterston^{3,9}



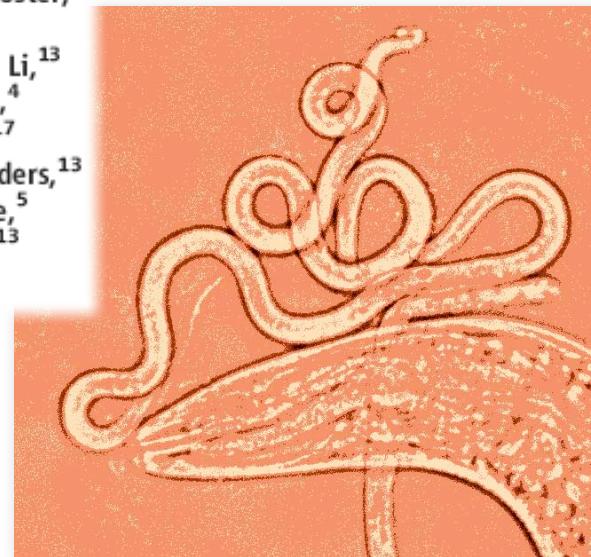


First Genome from a Parasitic Helminth

21 SEPTEMBER 2007 VOL 317 SCIENCE www.sciencemag.org

Draft Genome of the Filarial Nematode Parasite *Brugia malayi*

Elodie Ghedin,^{1,2}# Shiliang Wang,² David Spiro,² Elisabet Caler,² Qi Zhao,² Jonathan Crabtree,² Jonathan E. Allen,^{2,*} Arthur L. Delcher,^{2,†} David B. Giuliano,³ Diego Miranda-Saavedra,^{4,‡} Samuel V. Angiuoli,² Todd Creasy,² Paolo Amedeo,² Brian Haas,² Najib M. El-Sayed,^{2,§} Jennifer R. Wortman,² Tamara Feldblyum,² Luke Tallon,² Michael Schatz,^{2,†} Martin Shumway,² Hean Koo,² Steven L. Salzberg,^{2,†} Seth Schobel,² Mihaela Pertea,^{2,†} Mihai Pop,^{2,†} Owen White,² Geoffrey J. Barton,⁴ Clotilde K. S. Carlow,⁵ Michael J. Crawford,⁶ Jennifer Daub,^{7||} Matthew W. Dimmic,⁶ Chris F. Estes,⁸ Jeremy M. Foster,⁵ Mehul Ganatra,⁵ William F. Gregory,⁷ Nicholas M. Johnson,⁹ Jinming Jin,¹⁰ Richard Komuniecki,¹¹ Ian Korf,¹² Sanjay Kumar,⁵ Sandra Laney,¹³ Ben-Wen Li,¹⁴ Wen Li,¹³ Tim H. Lindblom,⁸ Sara Lustigman,¹⁵ Dong Ma,⁵ Claude V. Maina,⁵ David M. A. Martin,⁴ James P. McCarter,^{6,16} Larry McReynolds,¹⁰ Makedonka Mitreva,¹⁶ Thomas B. Nutman,¹⁷ John Parkinson,¹⁸ José M. Peregrín-Alvarez,¹ Catherine Poole,⁵ Qinghu Ren,² Lori Saunders,¹³ Ann E. Sluder,¹⁹ Katherine Smith,¹¹ Mario Stanke,²⁰ Thomas R. Unnasch,²¹ Jenna Ware,⁵ Aguan D. Wei,²² Gary Weil,¹⁴ Deryck J. Williams,⁷ Yinhua Zhang,⁵ Steven A. Williams,¹³ Claire Fraser-Liggett,^{2||} Barton Slatko,⁵ Mark L. Blaxter,⁷ Alan L. Scott²³



Filarial Genome Project



WHO



TDR



World Bank

Steven Williams

Smith College

Mark Blaxter

Univ of Edinburgh

Barton Slatko

New England Biolabs

Alan Scott

Johns Hopkins

Reda Ramsey

Aim Shams

Tania Supali

Univ of Jakarta

Kunthala Jayaraman

Anna Univ



Nature Biotechnology 2008. 26:909.

Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*

Pierre Abad^{1–3}, Jérôme Gouzy⁴, Jean-Marc Aury^{5–7}, Philippe Castagnone-Sereno^{1–3}, Etienne G J Danchin^{1–3}, Emeline Deleury^{1–3}, Laetitia Perfus-Barbeoch^{1–3}, Véronique Anthouard^{5–7}, François Artiguenave^{5–7}, Vivian C Blok⁸, Marie-Cécile Caillaud^{1–3}, Pedro M Coutinho⁹, Corinne Dasilva^{5–7}, Francesca De Luca¹⁰, Florence Deau^{1–3}, Magali Esquibet¹¹, Timothé Flutre¹², Jared V Goldstone¹³, Noureddine Hamamouch¹⁴, Tarek Hewezi¹⁵, Olivier Jaillon^{5–7}, Claire Jubin^{5–7}, Paola Leonetti¹⁰, Marc Maglano^{1–3}, Tom R Maier¹⁵, Gabriel V Markov^{16,17}, Paul McVeigh¹⁸, Graziano Pesole^{19,20}, Julie Poulain^{5–7}, Marc Robinson-Rechavi^{21,22}, Erika Sallet^{23,24}, Béatrice Ségurens^{5–7}, Delphine Steinbach¹², Tom Tytgat²⁵, Edgardo Ugarte^{5–7}, Cyril van Ghelder^{1–3}, Pasqua Veronico¹⁰, Thomas J Baum¹⁵, Mark Blaxter²⁶, Teresa Bleve-Zacheo¹⁰, Eric L Davis¹⁴, Jonathan J Ewbank²⁷, Bruno Favery^{1–3}, Eric Grenier¹¹, Bernard Henrissat⁹, John T Jones⁸, Vincent Laudet¹⁶, Aaron G Maule¹⁸, Hadi Quesneville¹², Marie-Noëlle Rosso^{1–3}, Thomas Schiex²⁴, Geert Smant²⁵, Jean Weissenbach^{5–7} & Patrick Wincker^{5–7}

PNAS 2008. 105:14802.

Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism

Charles H. Opperman^{a,b,c}, David M. Bird^{a,b}, Valerie M. Williamson^d, Dan S. Rokhsar^e, Mark Burke^a, Jonathan Cohn^a, John Cromer^a, Steve Diener^{a,f}, Jim Gajan^a, Steve Graham^a, T. D. Houfek^{a,g}, Qingli Liu^{d,h}, Therese Mitrosⁱ, Jennifer Schaff^{a,j}, Reenah Schaffer^a, Elizabeth Scholl^a, Bryon R. Sosinski^{k,l}, Varghese P. Thomas^d, and Eric Windham^a



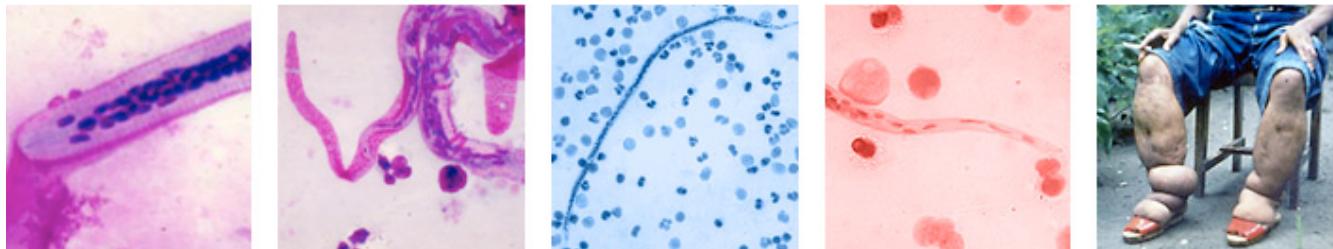
Helminth genome projects - 2009/2010

Genomic Sequencing Project Status				
Species	Common name	Genome Size (Mb)	Project	Status
<i>Schistosoma mansoni</i>	blood fluke	330	reference genome whole genome shotgun	Published
<i>S. haematobium</i>	blood fluke	270		
<i>Echinococcus multilocularis</i>	hydatid disease	150	reference genome whole genome shotgun whole genome shotgun	autoprefinishing/assembly planned in progress
<i>Echinococcus granulosus</i>	hydatid disease	150		
<i>Hymenolepis microstoma</i>	rodent tapeworm	?		
<i>Ascaris suum</i>	roundworm	230	reference genome	in progress
<i>Ascaris lumbricoides</i>	roundworm	230	1x capillary shotgun sequence	awaiting DNA
<i>Globodera pallida</i>		75-100	whole genome shotgun	in progress
<i>Haemonchus contortus</i>	barber-pole worm	60	reference genome	in progress
<i>Nippostrongylus brasiliensis</i>	model hookworm	?	reference genome	in progress
<i>Onchocerca volvulus</i>	river blindness	150	reference genome	in progress
<i>Strongyloides ratti</i>	threadworm	?	reference genome	in progress
<i>Strongyloides stercoralis</i>	human threadworm	?	whole genome shotgun	planned
<i>Teladorsagia circumcincta</i>		?	2x whole genome shotgun	in progress
<i>Trichuris muris</i>	model whipworm	96	reference genome	in progress
<i>Trichuris trichiura</i>	human whipworm	?	whole genome shotgun	planned



Helminth genome projects - 2010

Filarial worms Database

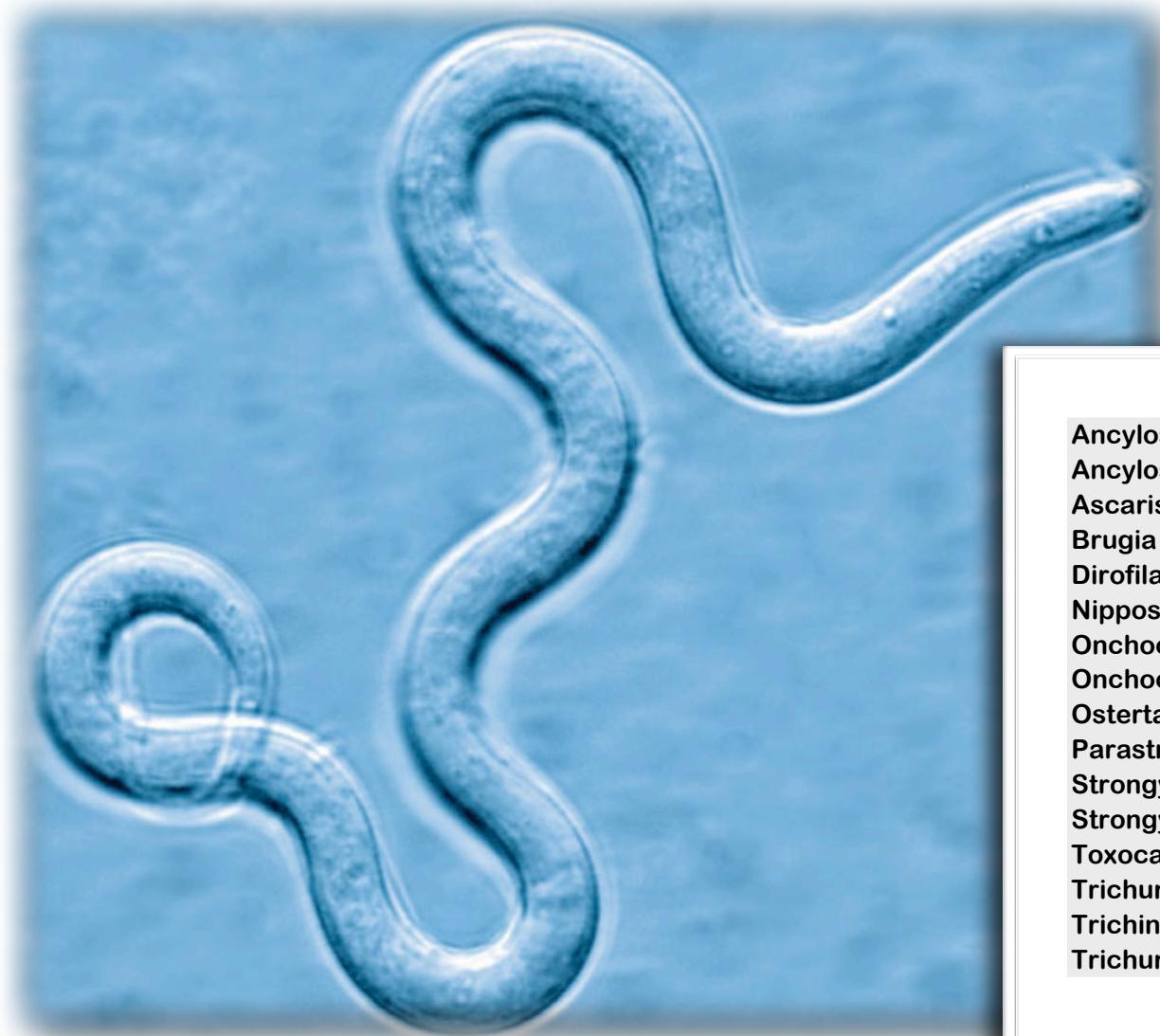


Sequence Downloads

	supercontigs.fasta
	contigs.fasta
	contigs.agp
	All File Types
Wuchereria bancrofti	↓ ↓ ↓ ↓
Onchocerca volvulus	↓ ↓ ↓ ↓
Brugia malayi	↓ ↓ ↓ ↓
Caenorhabditis elegans	↓ ↓ ↓ ↓
Wolbachia-Wuchereria bancrofti	↓ ↓ ↓ ↓
Wolbachia-Onchocerca volvulus	↓ ↓ ↓ ↓
Wolbachia-Brugia malayi	↓ ↓ ↓ ↓
Loa loa (V2)	↓ ↓ ↓ ↓
All Assemblies->	↓ ↓ ↓ ↓



Estimated Number of ESTs



SPECIES	# ESTS	CLADE
<i>Ancylostoma caninum</i>	94429	V
<i>Ancylostoma ceylanicum</i>	10590	V
<i>Ascaris suum</i>	451746	III
<i>Brugia malayi</i>	25678	III
<i>Dirofilaria immitis</i>	4005	III
<i>Nippostrongylus brasiliensis</i>	13452	V
<i>Onchocerca flexuosa</i>	2124	III
<i>Onchocerca volvulus</i>	1230	III
<i>Ostertagia ostertagi</i>	6558	V
<i>Parastrengyloides trichosuri</i>	7963	IVa
<i>Strongyloides ratti</i>	14761	IVa
<i>Strongyloides stercoralis</i>	11335	IVa
<i>Toxocara canis</i>	4370	III
<i>Trichuris muris</i>	4388	I
<i>Trichinella spiralis</i>	25049	I
<i>Trichuris vulpis</i>	3063	I
TOTAL	680741	

Table based on Table 1: Progress of Nematode EST Sequencing at the Genome Sequencing Center, Washington Univ. (Updated 07-01-08) at Nematode.net

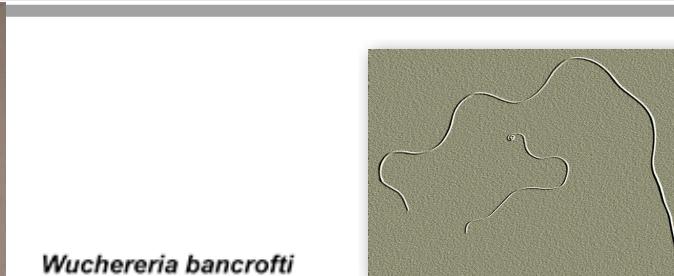


Features of nematode genomes

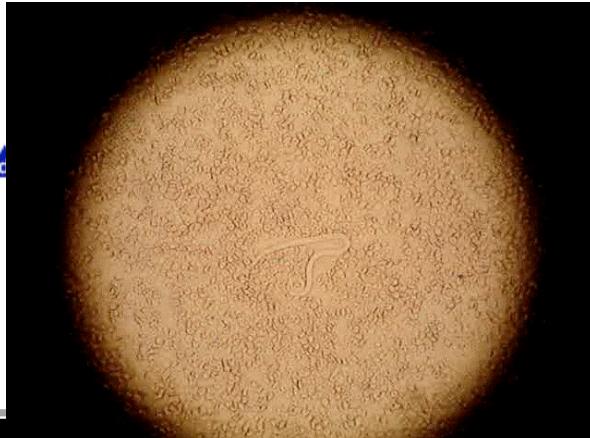
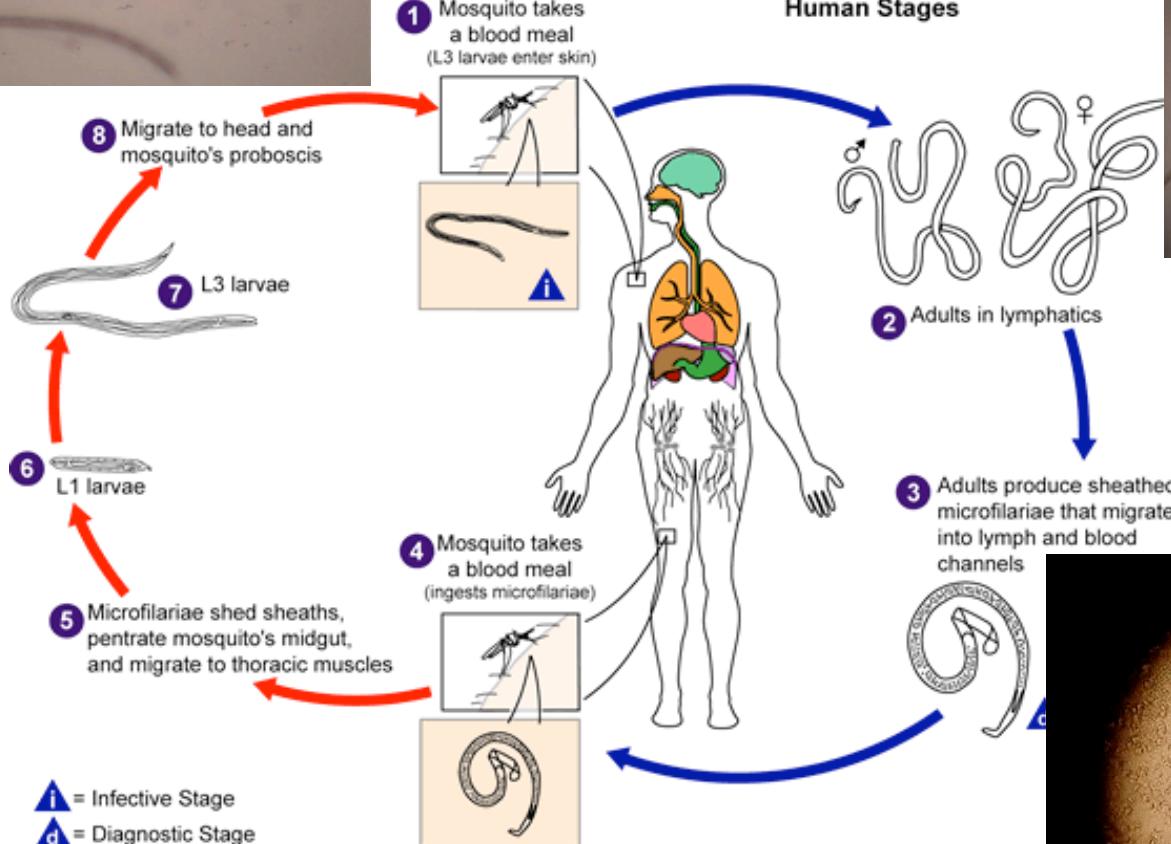
	<i>C. elegans</i>	<i>C. briggsae</i>	<i>P. pacificus</i>	<i>B. malayi</i>	<i>M. incognita</i>	<i>M. hapla</i>	<i>S. japonicum</i>
Genome size (Mb)	100	104	142	98	86	54	398
No. chromosomes	6	6	6	5	vary	16	9
G+C content	35.5%	37.4%	42%	30.5%	31.4%	27.4%	34%
Number of protein models	23,662	19,934	29,201	16,500	19,212	14,420	13,469
Gene density (genes/Mb)	236	192	206	162	223	270	34
repeat rate	18%	19%		15%	18%	18%	40%



Filarial Nematodes



Human Stages





Bm genome in pieces

5 chromosome pairs in 8250 bits!
Longest scaffold: 6.5 Mb

(*C. elegans* chromosomes range from 14 Mb to 21 Mb)



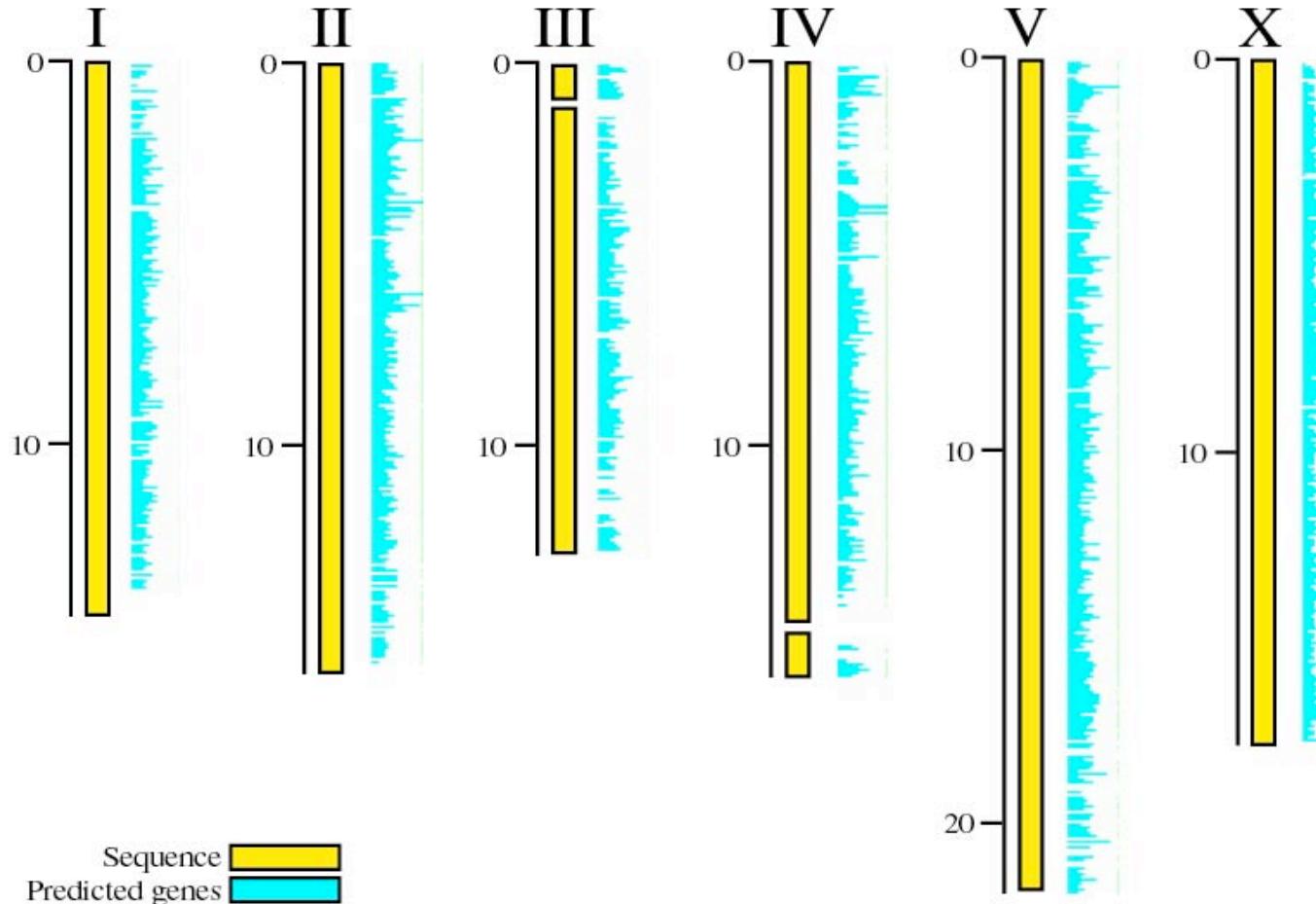
Sakaguchi et al. (1983) J Parasitol 69:1090

Bm Genome Project Update:

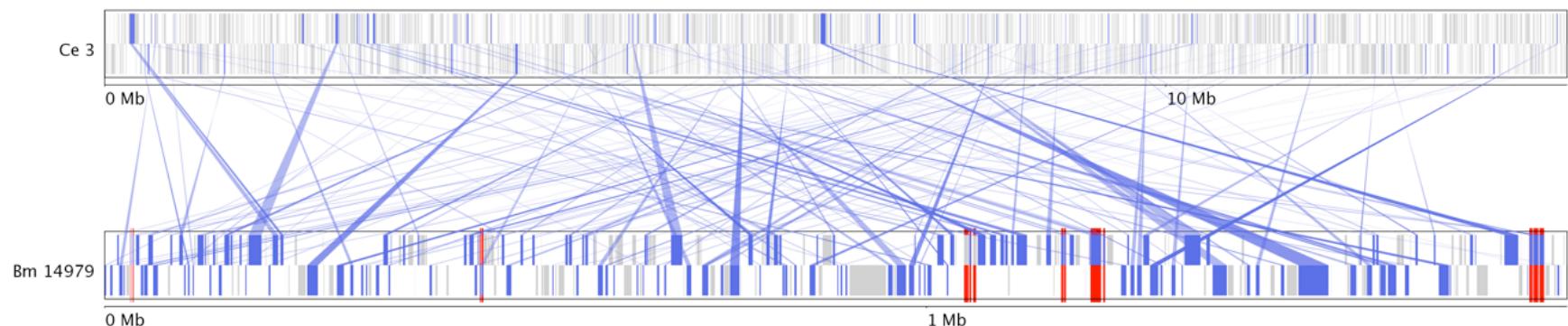
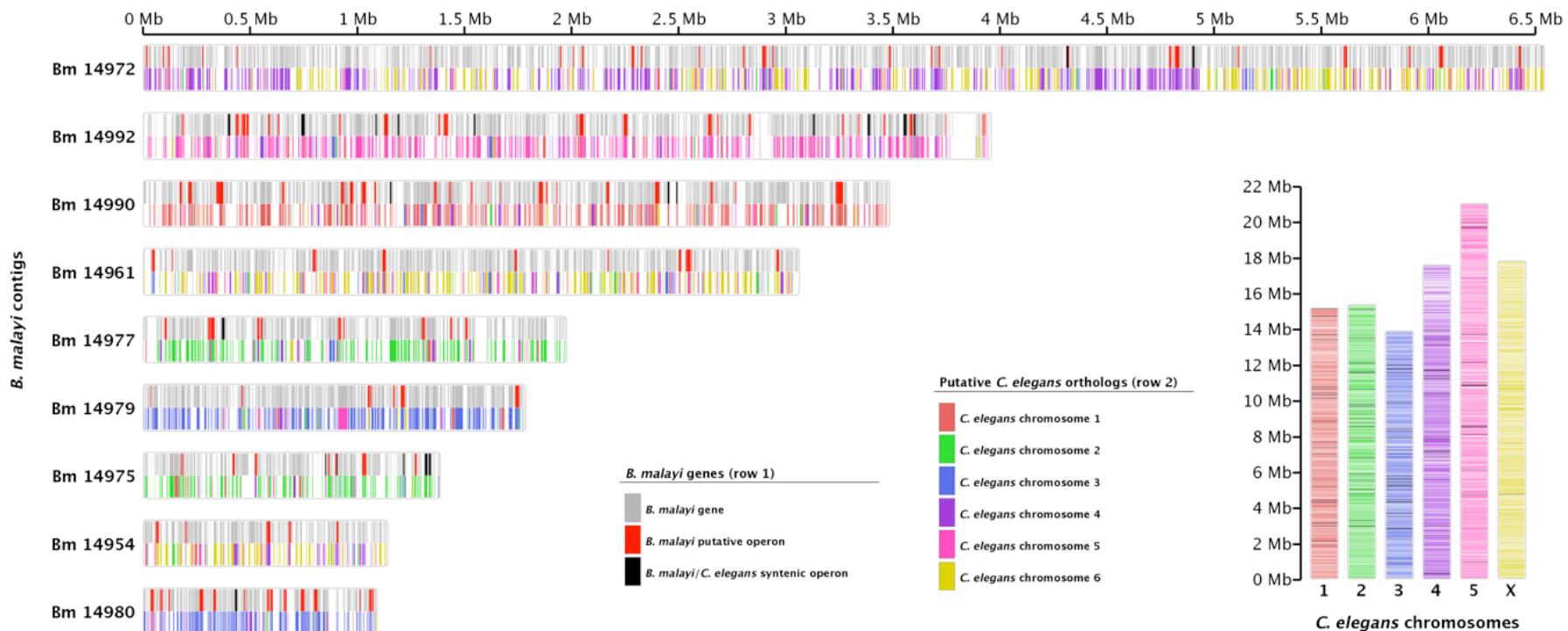
- 20 kb and 3 kb paired-end genomic libraries are being sequenced on via 454 and Illumina at Pitt by Elodie Ghedin
- stage-specific cDNA libraries of embryos, male, female, L3, L4, early mf and late mf (made by Shelly Michalski, U Wisconsin, Oshkosh) are being sequenced via Solexa by Matt Berriman (Sanger) to aid in annotation



C. elegans chromosomes



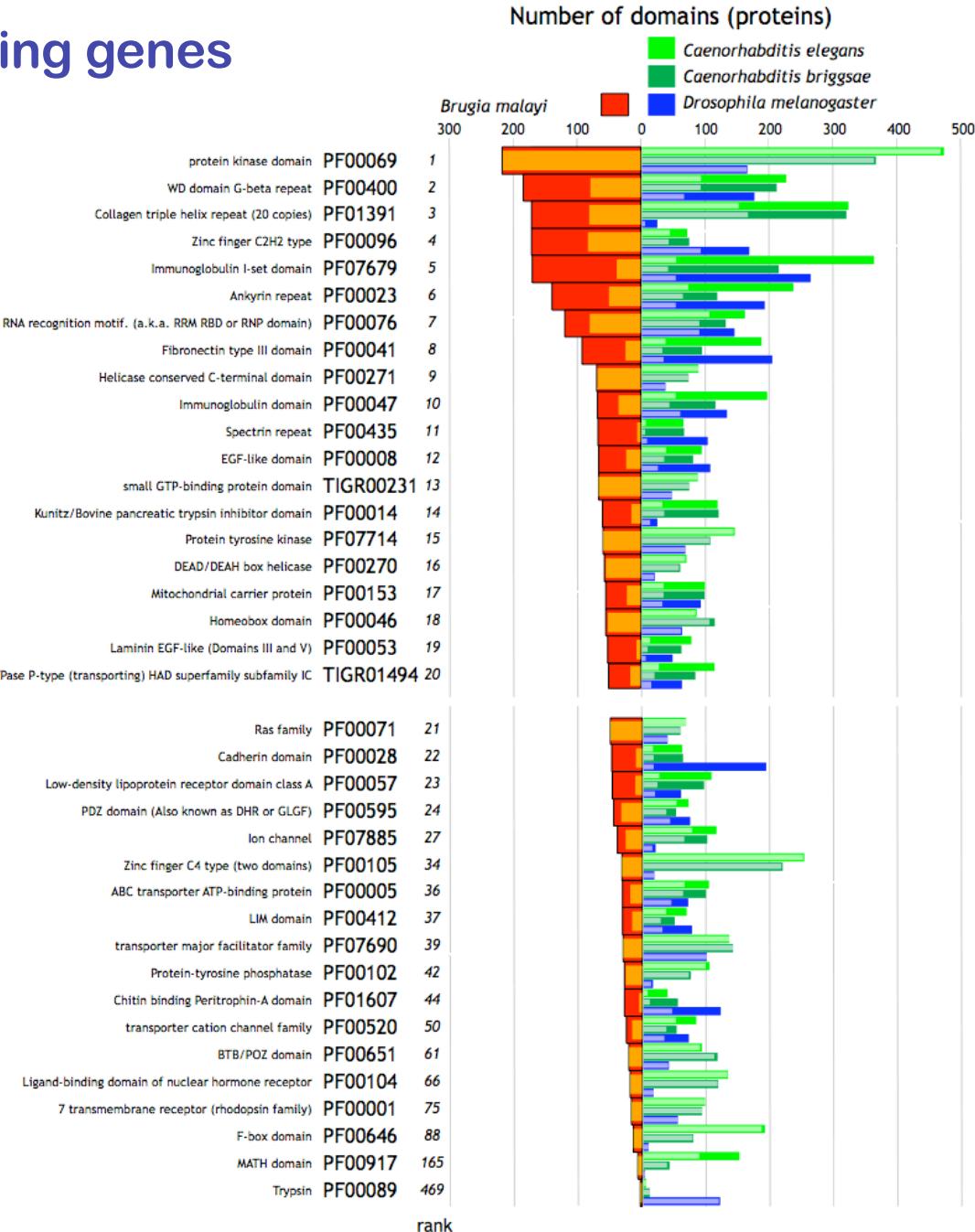
There is conservation of linkage but not synteny between *B. malayi* and *C. elegans*



Total number of Protein-coding genes

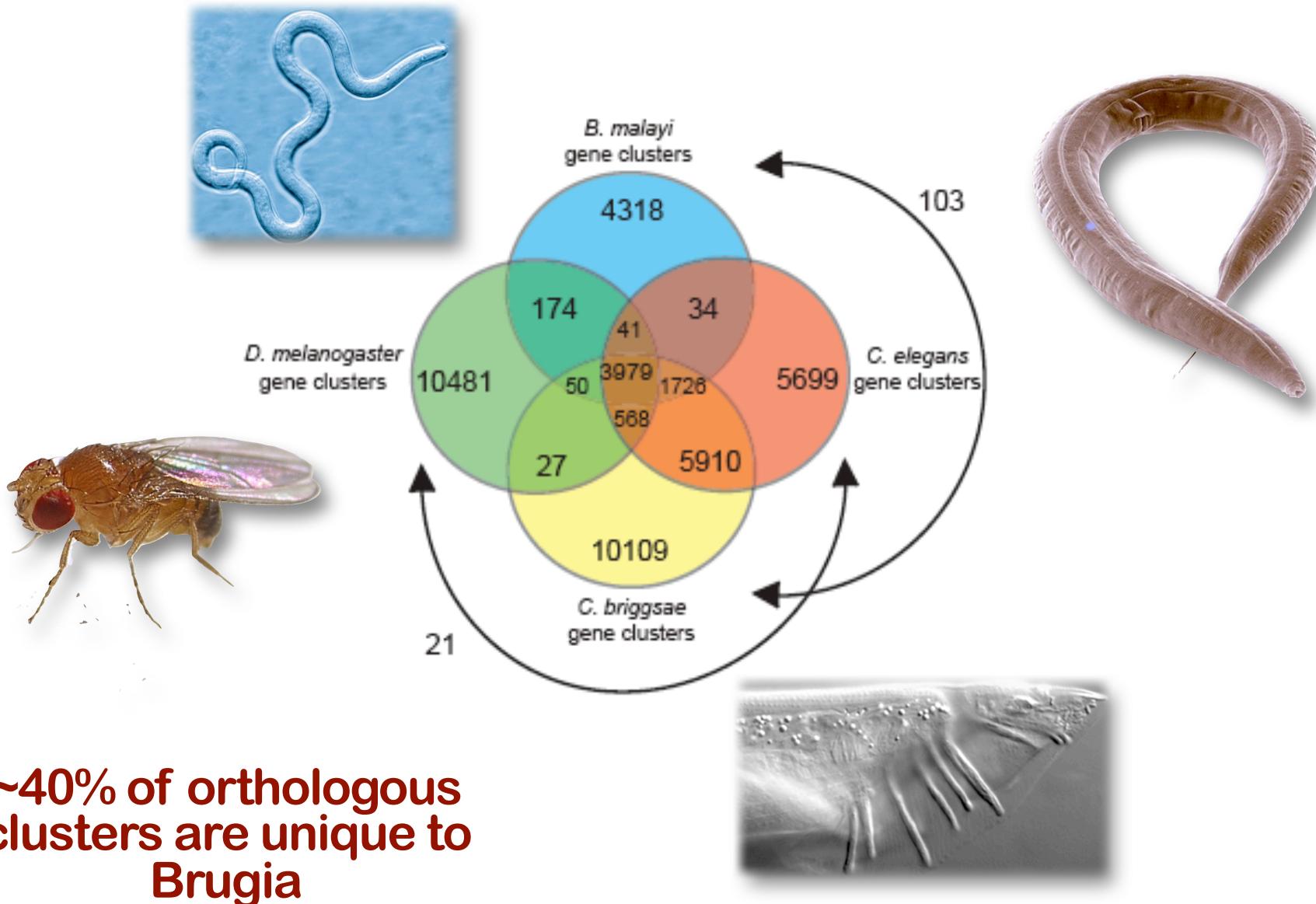
B. malayi	16,500 (est)
C. elegans	23,662
C. briggsae	19,507
D. melanogaster	13,792

Much of the difference between Ce and Bm can be accounted for by expanding the number of genes within certain gene families in Ce



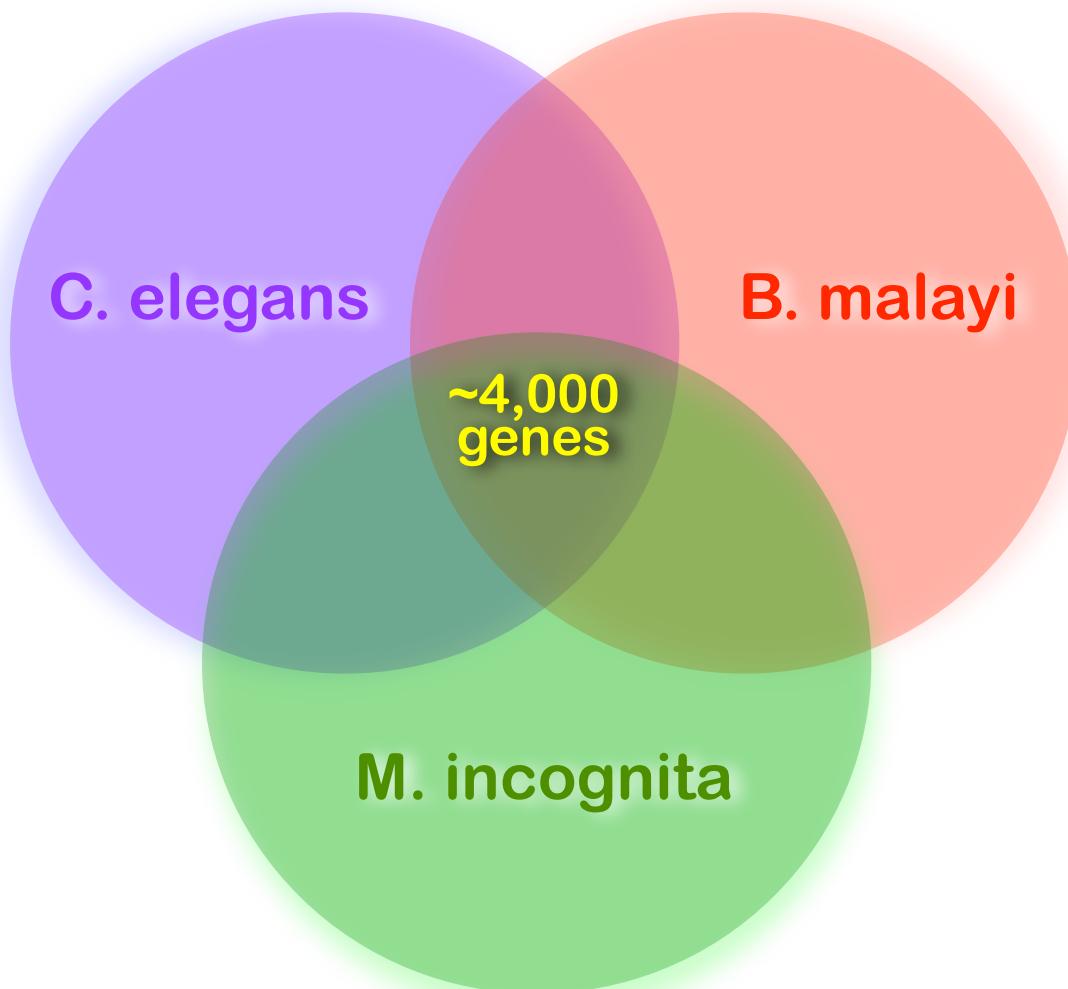


Core genes





Core nematode genes - circa 2009





trans-splicing in nematodes

A majority of nematode mRNAs have at their 5' end a conserved 22 nt leader sequence

The spliced leader (SL) sequence is trans-spliced to the 5' end of genes from an SL RNA of ~100 nt

In contrast to filarial nematodes which have only a single species of SL, *C. elegans* has as ~20 different SL genes

The identification of multiple SLs in Ce lead to the observation that as much as 15% of the genes are organized into **operons**

Polycistron on chromosome

Gene A



Gene B

Primary transcript

Intergenic
spacer

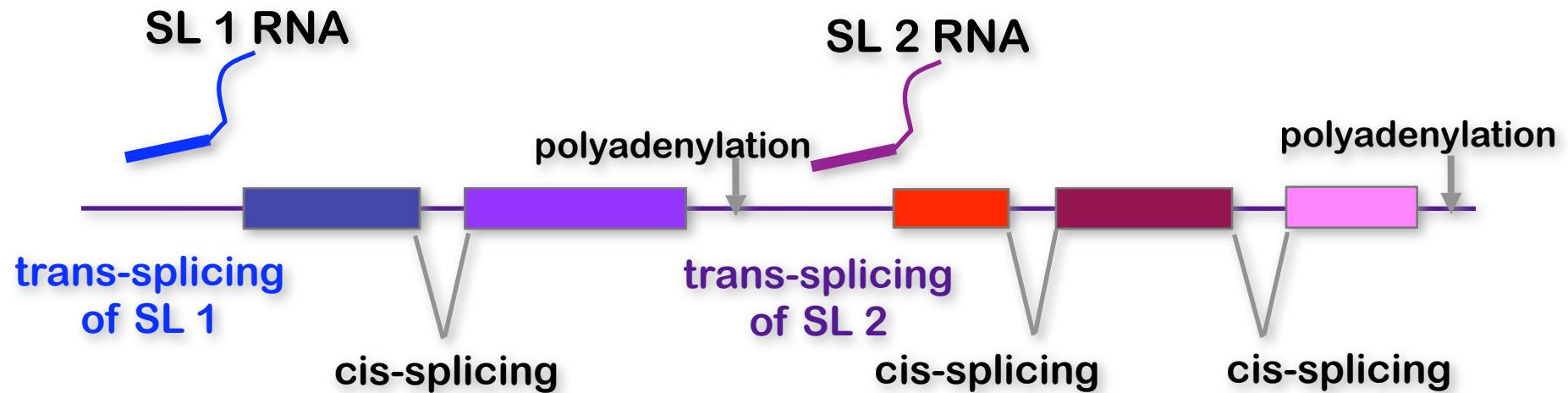


outron

intron

intron

intron



Gene A mRNA

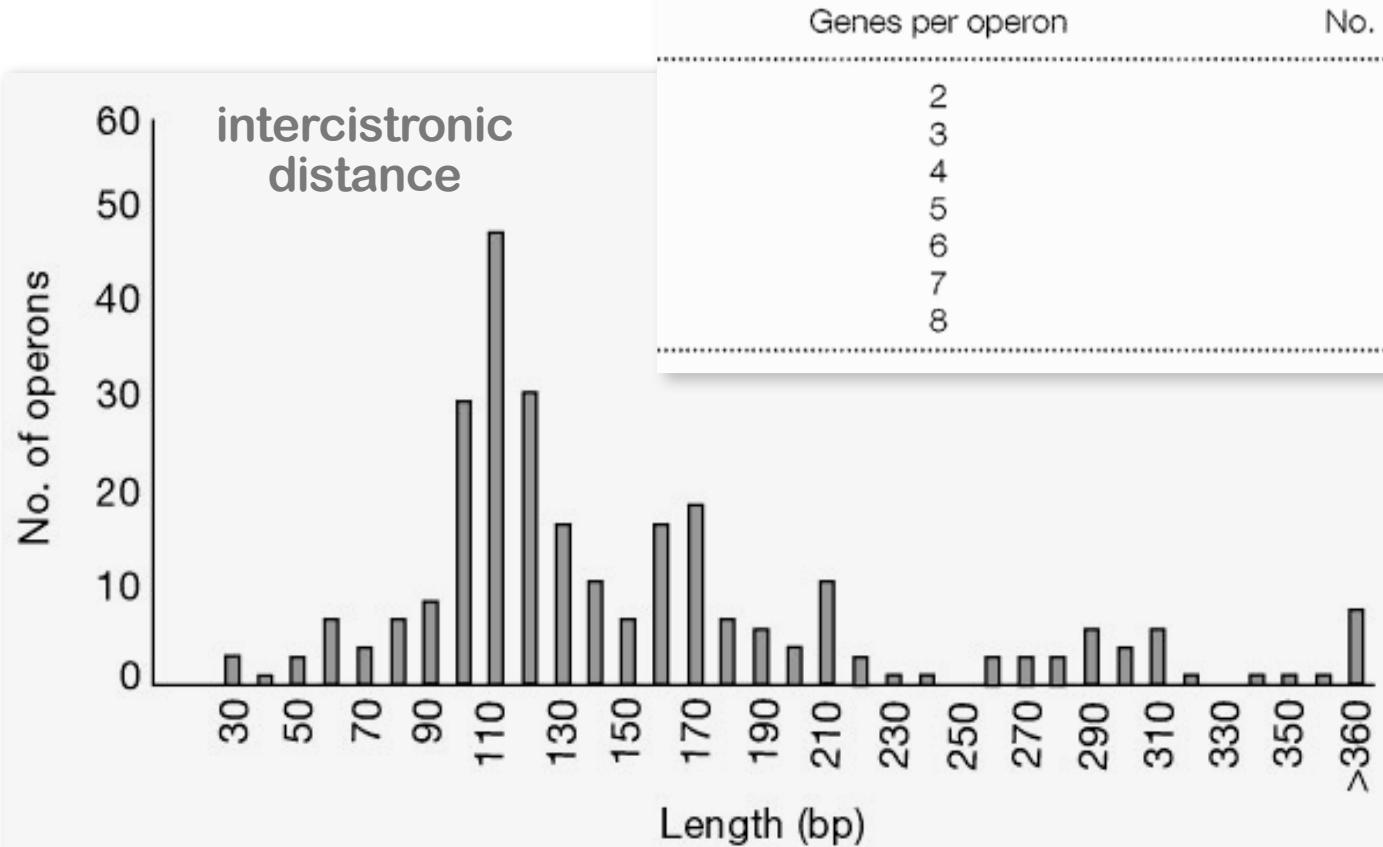


Gene B mRNA





Operon intercistronic distances

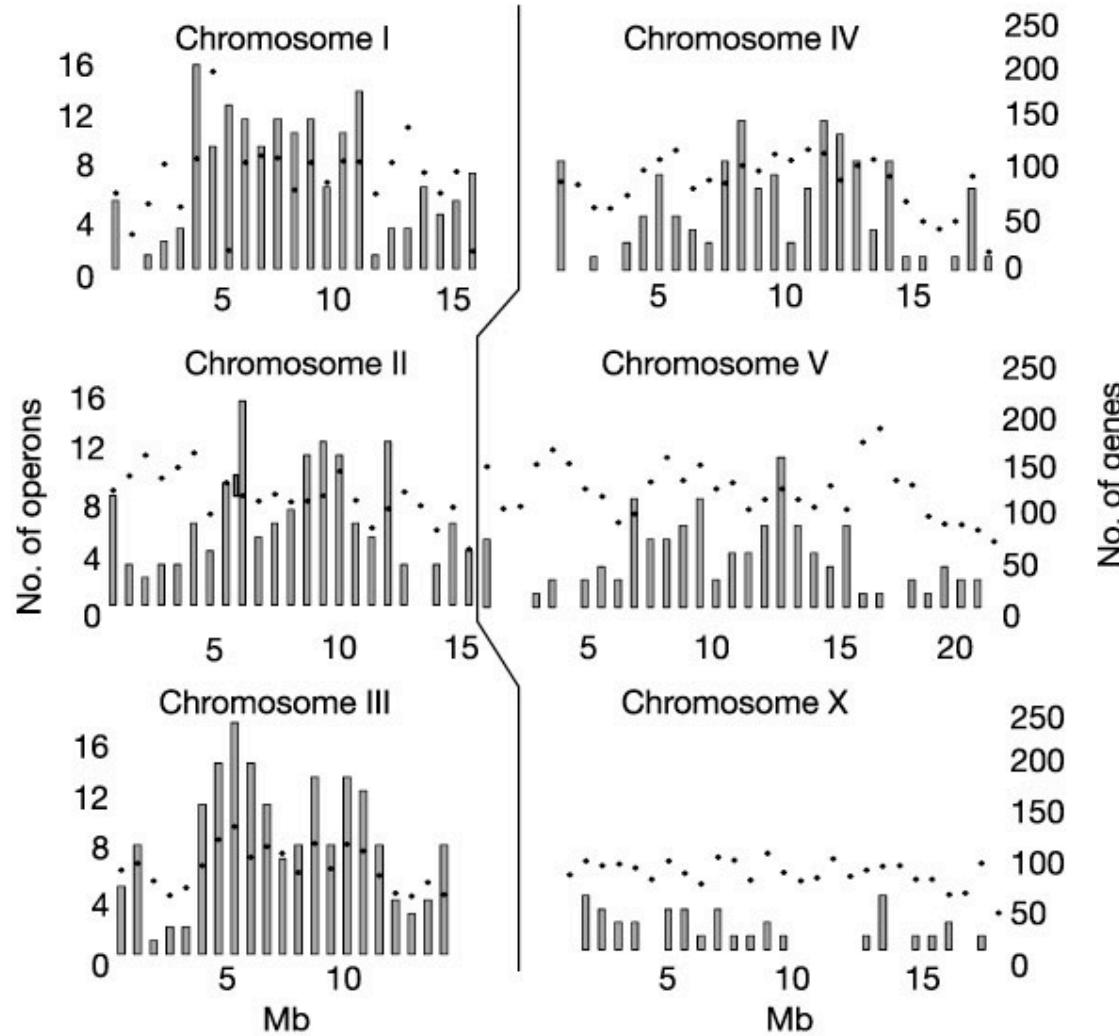


The number of genes per operon

Genes per operon	No. of operons
2	549
3	207
4	75
5	33
6	13
7	3
8	1

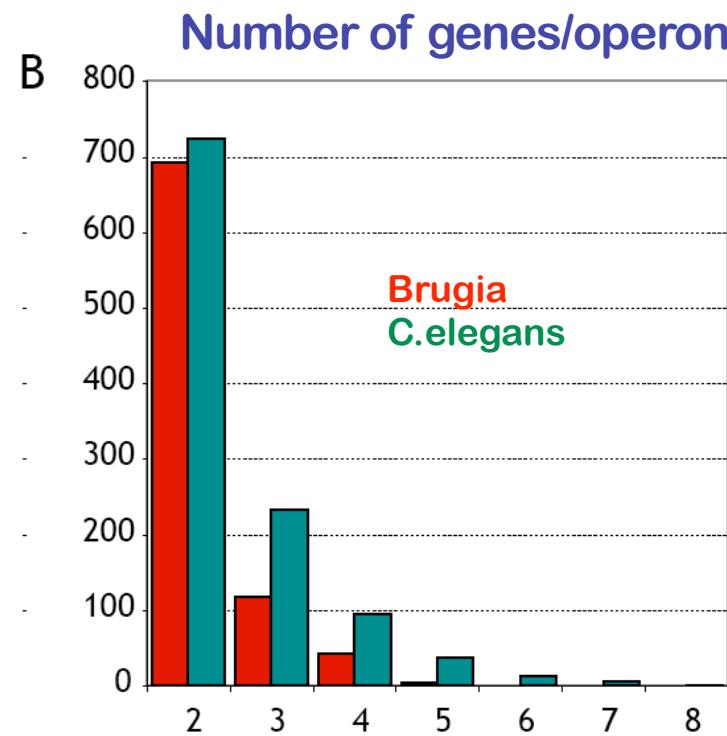
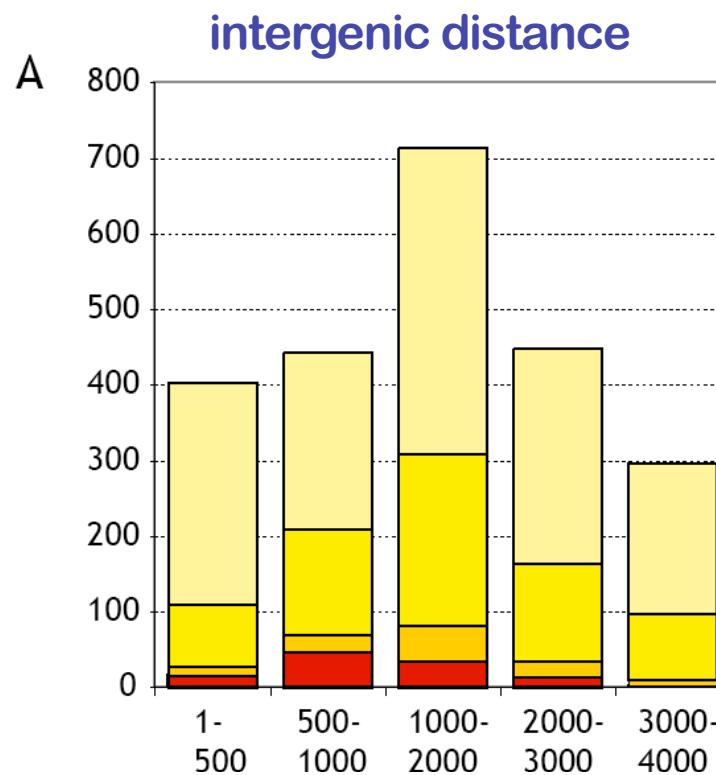


Operons on *C. elegans* chromosomes





Putative Operons in *B. malayi*



Longer intergenic distance than Ce
Number of genes/operon about the same as Ce



B. malayi Genomics



Nuclear genome
98 Mb

~12% repeats (30,000 copies of 322 bp Hha I repeat)

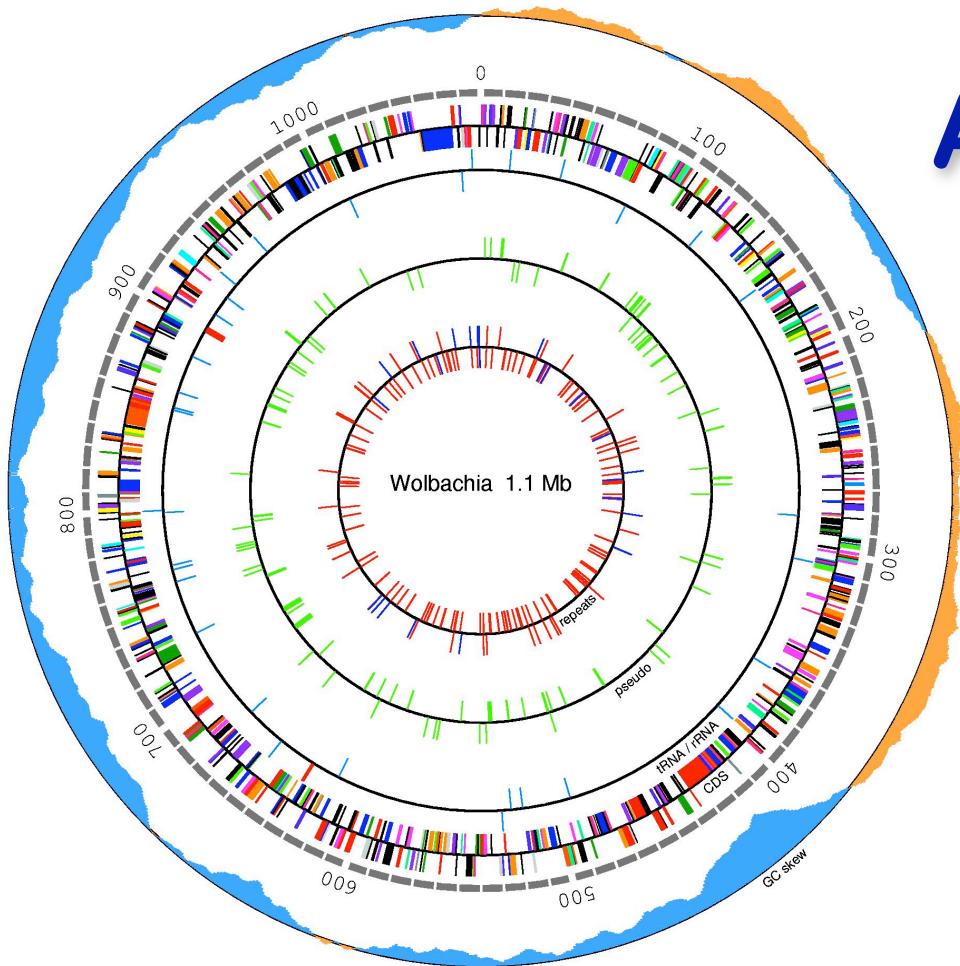
Mitochondrial genome

~14 Kb
12 protein coding genes, 2 rRNAs, 22 tRNAs

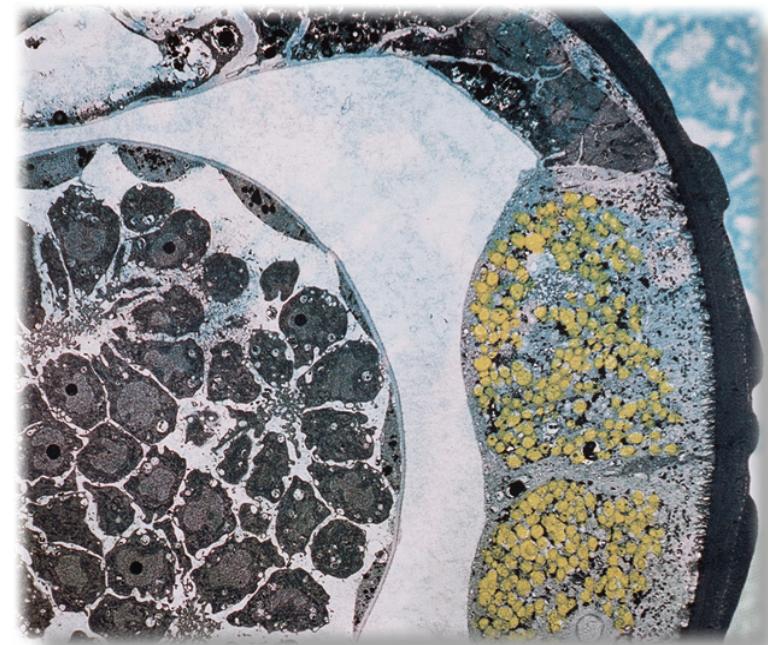
The Third genome
1.08 Mb



The Endosymbiont Genome



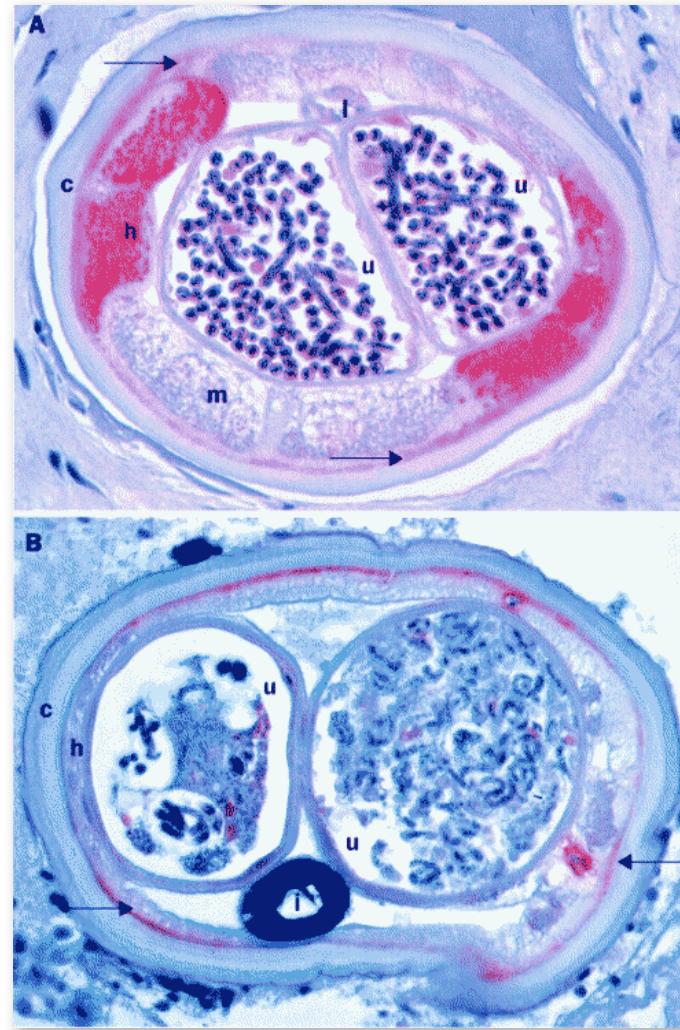
~1.08 Mb
AT content = 50%



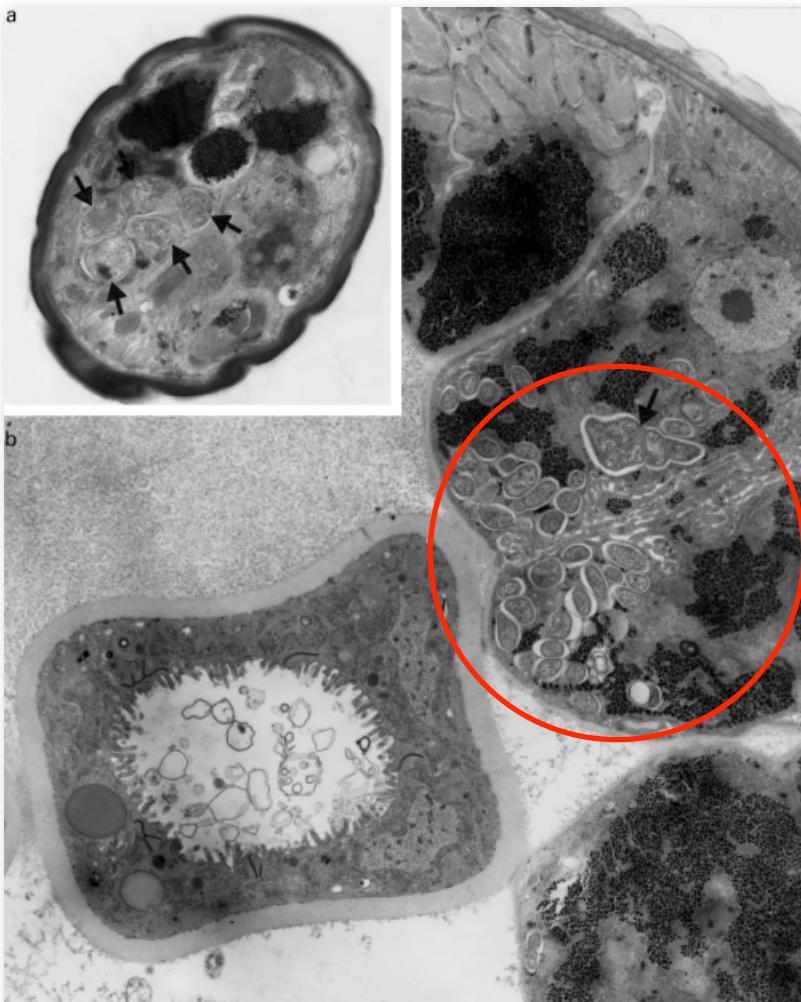


Wolbachia found in several cell types

midbody cross-sections of adult
Onchocerca volvulus
female



Hoerauf et al. 2000. Lancet. 355(9211):1242-3.



The number of bacteria increases dramatically during the development from ~100/larvae to >10⁷/adult

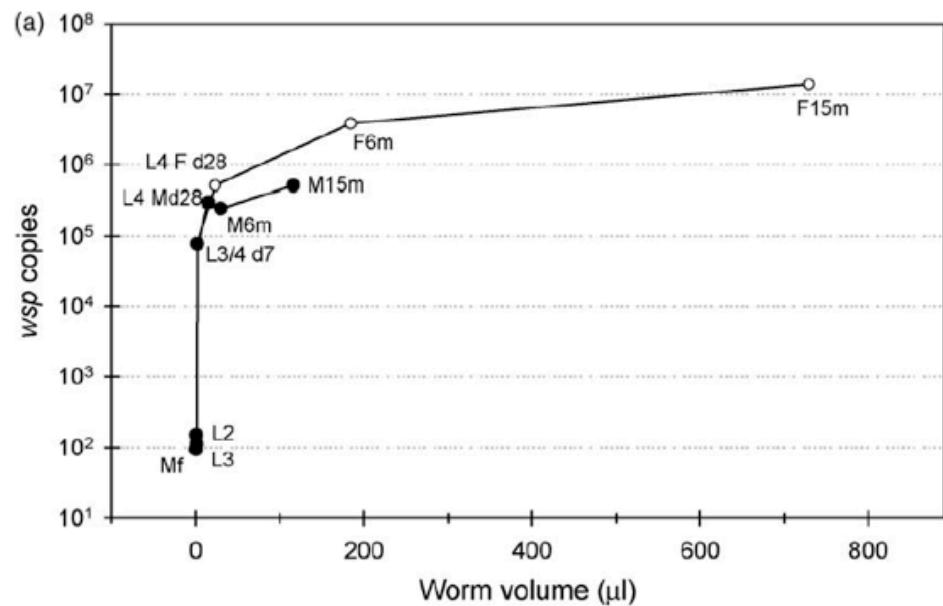


Figure 2. The relationship of *Bm-Wolbachia* numbers with worm volume. Copies of *wsp* plotted against the volume of *B. malayi* at each stage. Mf = microfilariae (L1); M = male; F = female; d = day; m = month. From McGarry et al. 2004. MBP 135:57-67.



Selection pressure and the Bm-Wolbachia genome

The 1.08 Mb Bm-Wolbachia genome is smaller than the Wolbachia from *Drosophila* (wMel, 1.27 Mb) and the related *Rickettsia prowazekii* (1.11 Mb).

Genes lost

- the genes required to infect new hosts
- the genes encoding molecules used by other bacteria to evade the host immune responses
- several genes encoding for DNA repair, for RNA modification and for cross-linking and degrading the carbohydrate backbone of peptidoglycans.
- the ~20 genes required for the biosynthesis of LPS

Genes retained

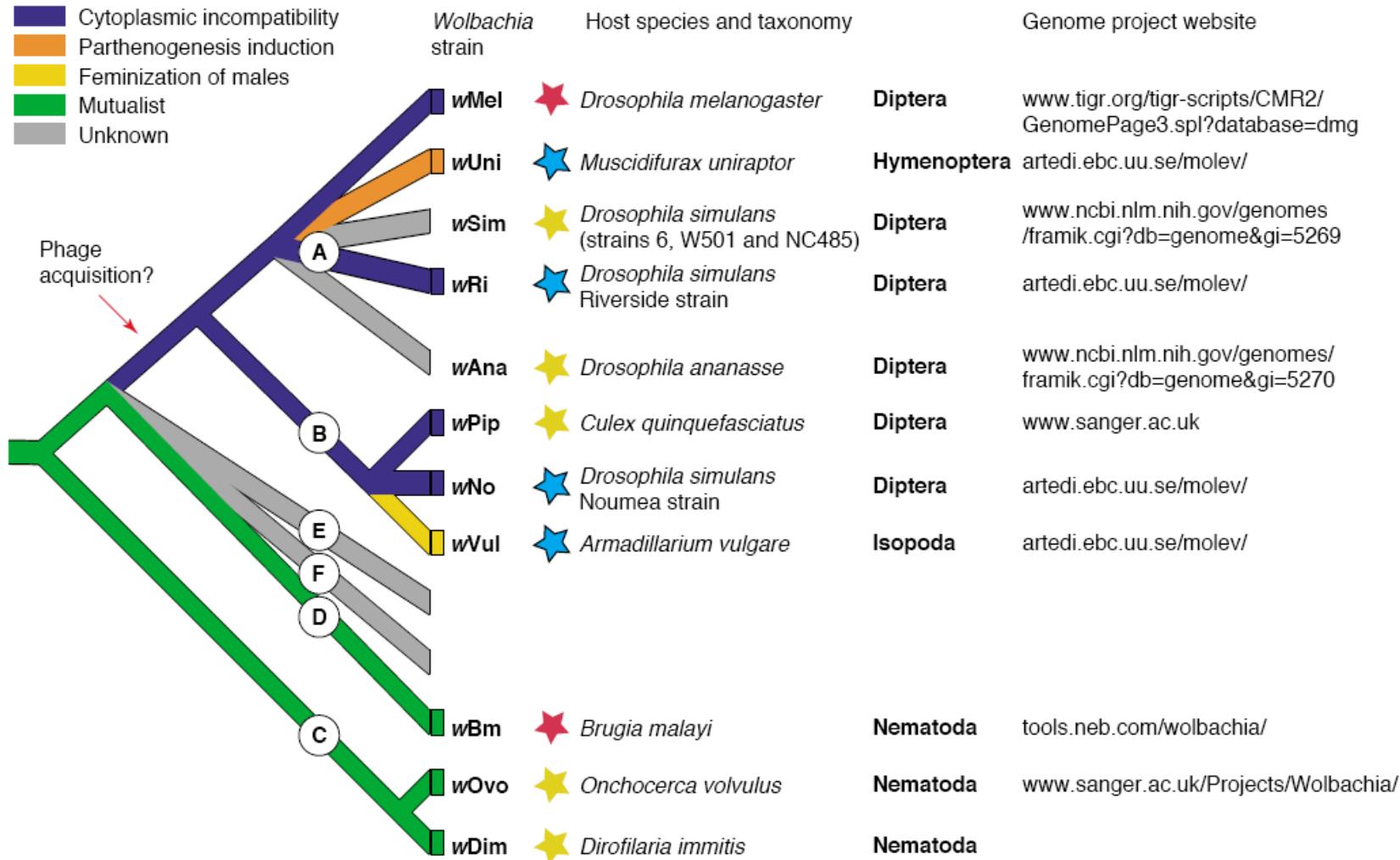
- full complement of genes encoding the molecules necessary for the synthesis of the co-factors riboflavin, flavin adenine dinucleotide, and heme
- in contrast to a majority of endosymbiotic bacteria, the genes necessary to synthesize all nucleotides (except one)
- Bm-Wolbachia has an intact type IV secretion system



Wolbachia is a mutualist in filarial worms

Symbiont–host interaction

- [Purple square] Cytoplasmic incompatibility
- [Orange square] Parthenogenesis induction
- [Yellow square] Feminization of males
- [Green square] Mutualist
- [Grey square] Unknown





Meloidogyne hapla

hydroxymuconic semialdehyde hydrolase

endoglucanases

chorismate mutase

exo-polygalacturonase

glutamine synthetase

isochorismatase

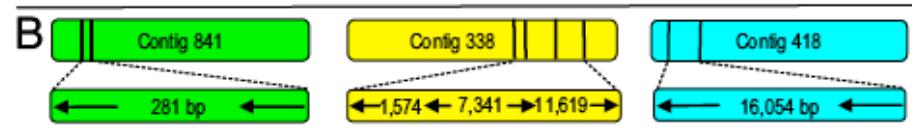
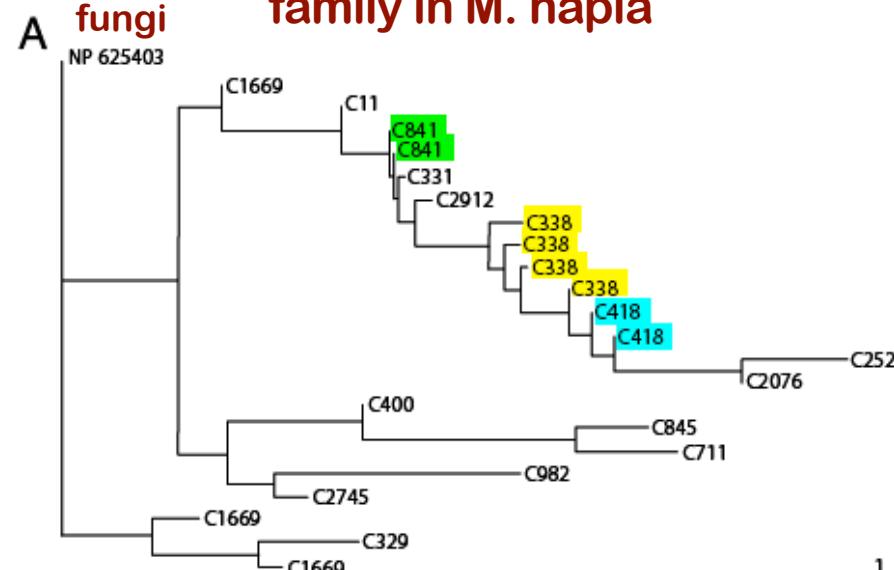
L-threonine aldolase

NodL

pectate lyase

other pectinases

the 22 member pectate lysae gene family in *M. hapla*



Sequencing Challenges

lack of clonal populations

No cell lines

No viruses

No tumors



WormBook

THE ONLINE REVIEW OF *C. elegans* BIOLOGY



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Nematode.net
Genome Sequencing Center

<http://www.nematode.net/index.php>

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Genome of
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