

COMPLEX HYBRID ORIGINS OF ROOT KNOT NEMATODES

Dave Lunt

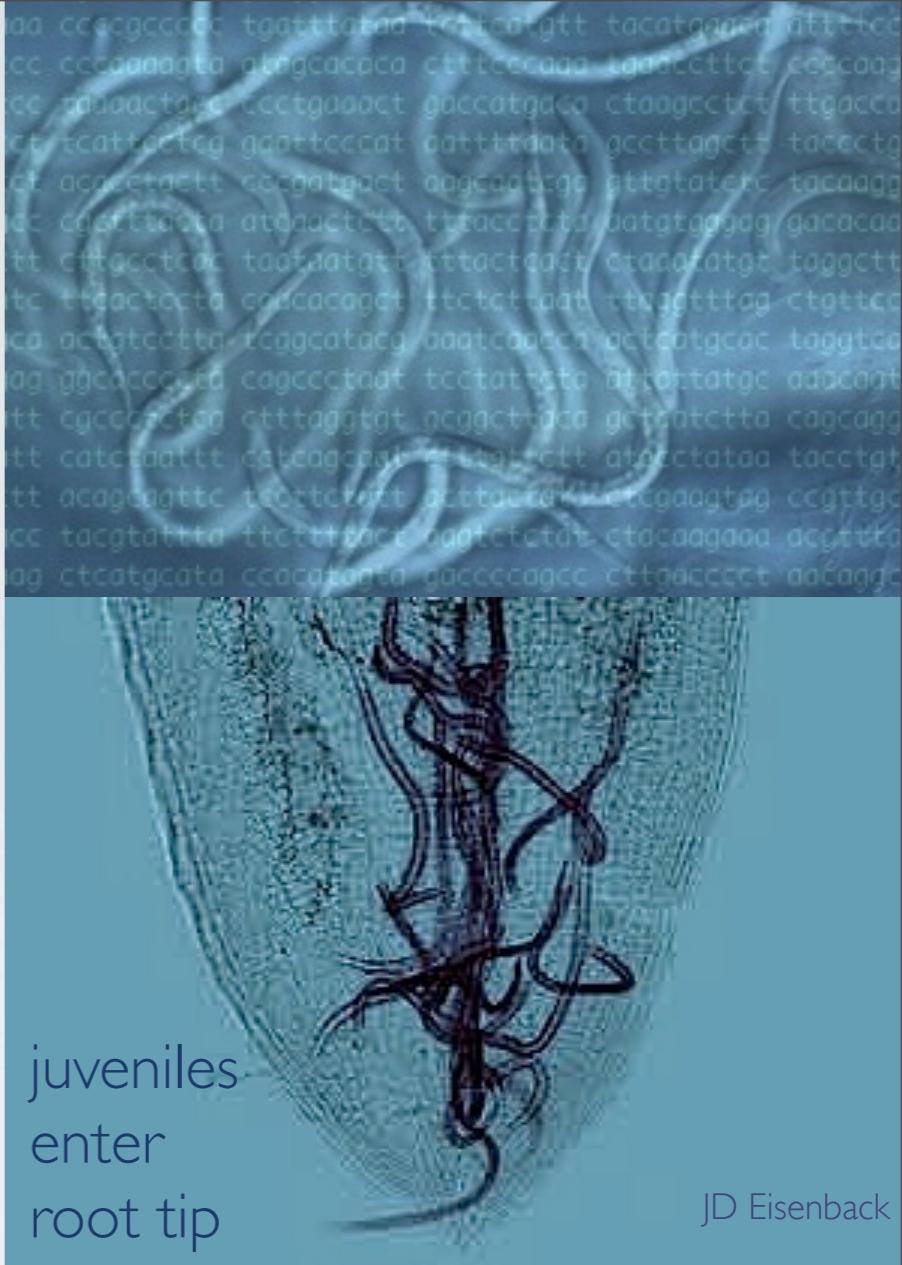
Evolutionary Biology Group, University of Hull

Sujai Kumar

Georgios Koutsovoulos

Mark Blaxter

Institute of Evolutionary Biology, University of Edinburgh



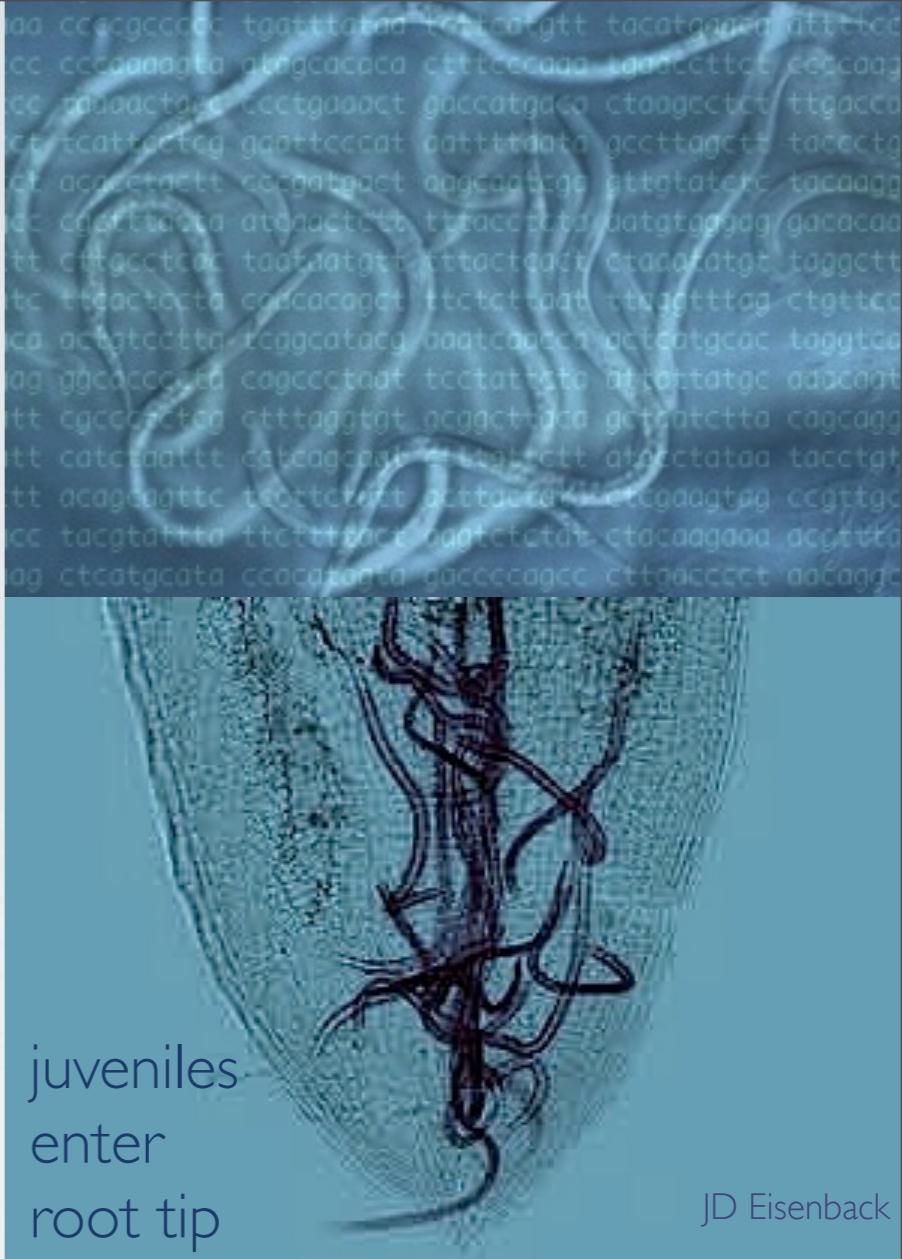
COMPLEX HYBRID ORIGINS OF ROOT KNOT NEMATODES

Dave Lunt
Evolutionary Biology Group, University of Hull

 @EvoHull  +EvoHull

 davelunt.net  dave.lunt@gmail.com

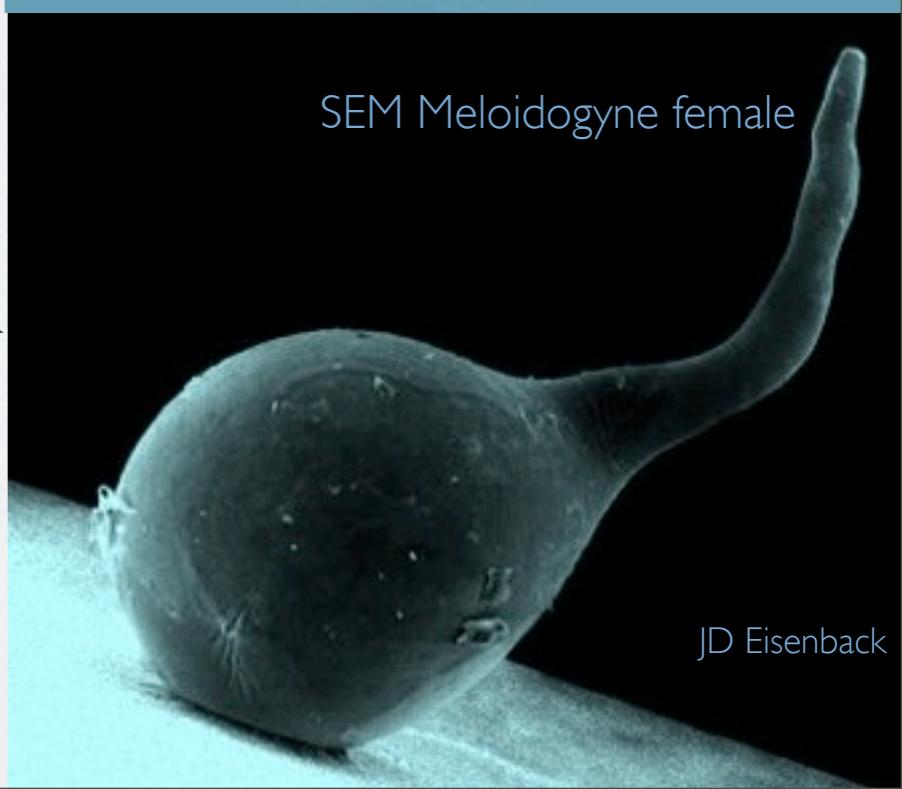
 @davelunt  +davelunt



Mark Blaxter

Institute of Evolutionary Biology, University of Edinburgh

 nematodes.org  mark.blaxter@ed.ac.uk



<http://www.slideshare.net/davelunt/lunt-nottingham>



<http://www.github.com/davelunt>

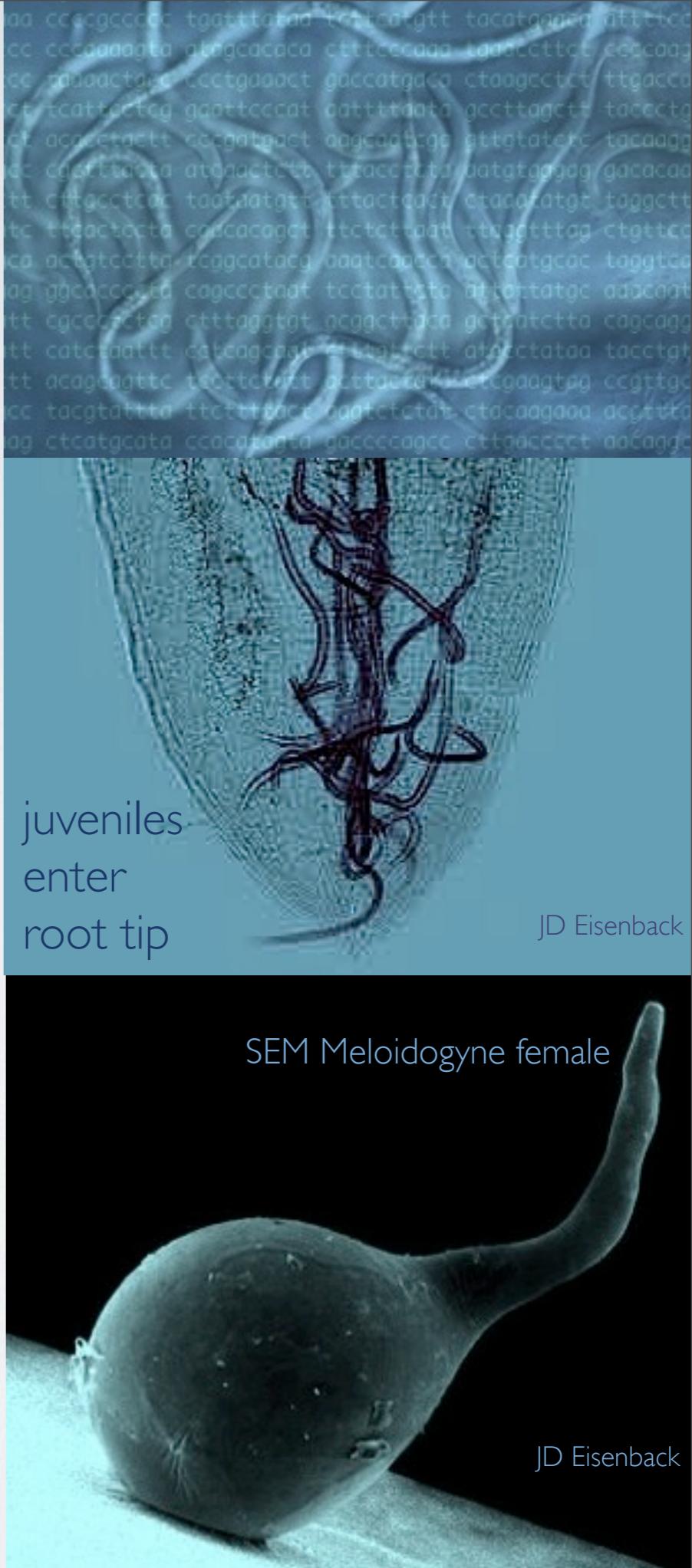
COMPLEX HYBRID ORIGINS OF ROOT KNOT NEMATODES

Acknowledgements

Africa Gómez, Richard Ennos, Amir Szitenberg,
Karim Gharbi, Chris Mitchell, Steve Moss, Tom
Powers, Janete Brito, Etienne Danchin, Marian
Thomson & GenePool

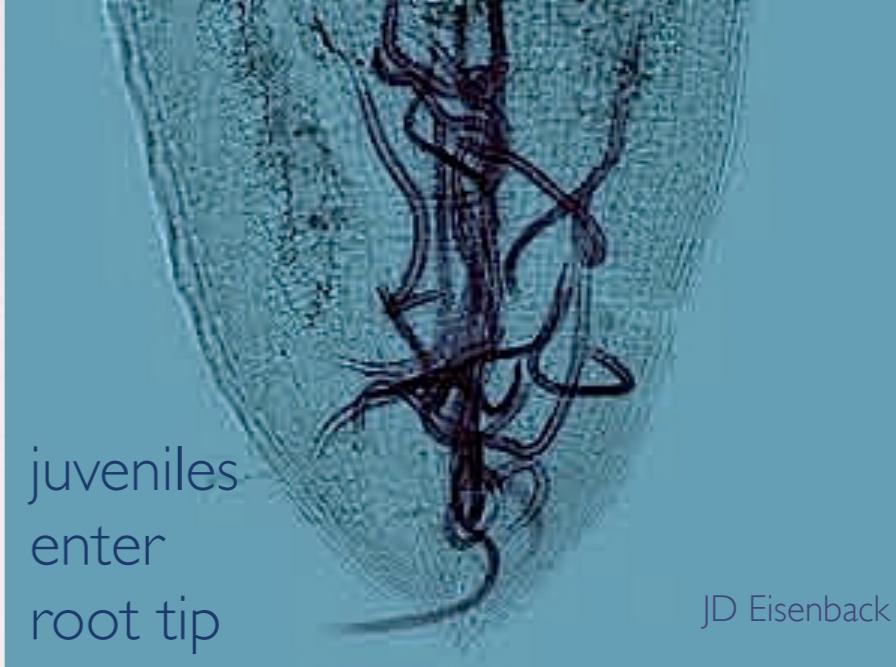
Funding

NERC, BBSRC, Yorkshire Agricultural Society,
Nuffield Foundation, University of Hull,
University of Edinburgh



WHAT'S IN A GENOME & WHY?

In many eukaryotes its
mostly transposons,
repeats, & sequences
of *incertae sedis*



juveniles
enter
root tip

JD Eisenback



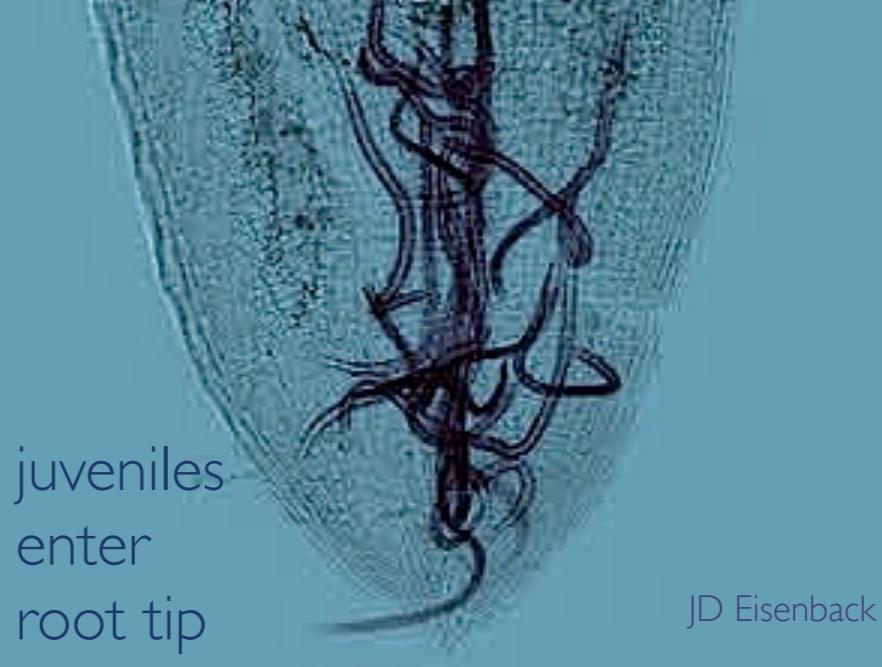
SEM Meloidogyne female

JD Eisenback

WHAT'S IN A GENOME & WHY?

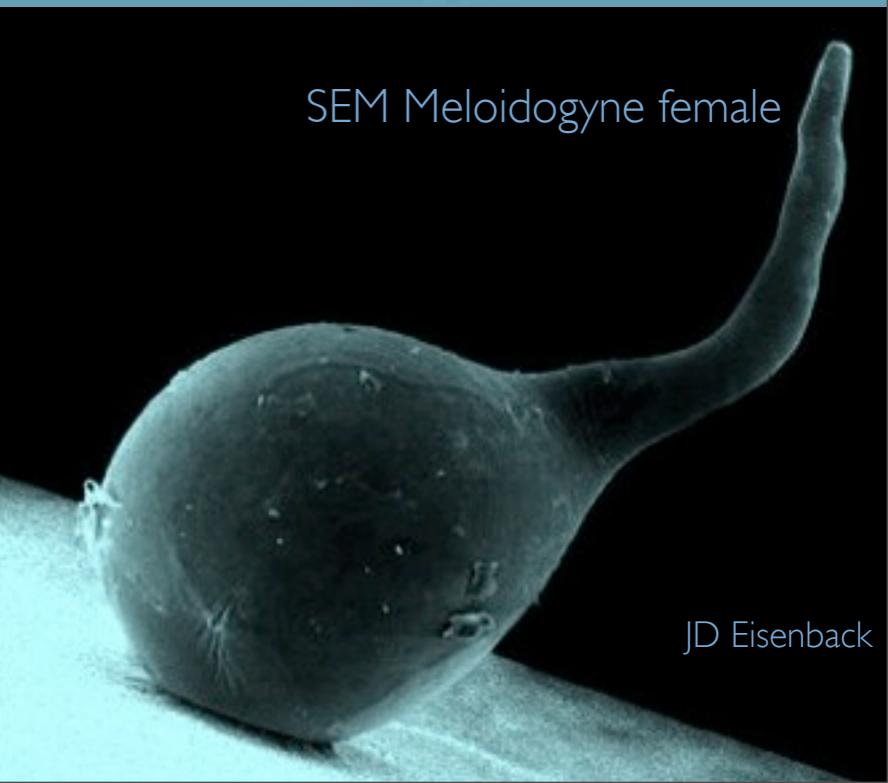
In many eukaryotes its
mostly transposons,
repeats, & sequences
of *incertae sedis*

But Why?



juveniles
enter
root tip

JD Eisenback



SEM Meloidogyne female

JD Eisenback

WHAT'S IN A GENOME & WHY?

Evolutionary Forces:

- Selection
- Gene Flow
- Mutation
- Drift
- Recombination

aa cccgcggcc tgatgtataa tttatgtt tacatgggg attttcc
cc cccggatgtt gtcgcacaca cttcccaaa tttttttttt ccccaag
cc ggaaacttgc ccctgaaact gaccatgaca ctaagccctt ttgaccc
ct tcatttcgt gatttcccat cattttataa gccttagctt tacatgt
ct acatctactt ccggatactt agggatcggg ttgttatctc tacaagg
tc cgtttataa ataaacttcc ttatccctt atatgtggdg gacacaa
tt cttgcctcac taataatgtt ttatctactt ctatataatgt taggttt
tc ttcaactcttcc cccacatgtt ttctcttcat ttaggttttag ctgttcc
ca actatcccttcc tccgcatacg aaatccatcc tttatgcac taggttt
ag ggcacccatc cagccctaat tcctatccat ttatccatgc adccat
tt cggccatcg ctttaggtgtt cccgcttccat gctatcttcc cagcagg
tt catccatccat cttccatgcat tttatccat tccgaatgtt ccgttgt
tt acageggttc tccctcttcc tttatccat tccgaatgtt ccgttgt
cc tacgtattttcc ttctttccat tttatccat tttatccat tttatccat
ag cttatgcata ccacatccat gccccccatcc ctttccatcc tttatccat



WHAT'S IN A GENOME & WHY?

Evolutionary Forces:

Selection
Gene Flow
Mutation
Drift

Recombination

aa cccgcggcc tgatttataa tttatgtt tacatgggg attttcc
cc cccggatgtt gtcgcacaca ctttccggg tttttttttt ccccaag
cc ggaaactggc ccctggaaact gaccatgaca ctaaggccctt ttggcc
ct tcatttcgg gatttcccat cattttataa gccttagctt tacatgt
ct acatctactt ccggatactt agggatcggtt ttgttatctc tacaagg
tc cgtttataa ataaaaatctt ttatccctt atatgtggdg gacacaa
tt ctgtccctcc taaaatgtt ttatctactt ctatatatgt taggttt
tc ttcaactcttcc cccacatgtt ttctcttcat ttaggttttag ctgttcc
ca actatcccttcc tccgcatacg aaatccatcc tttatgcac taggttt
ag ggcacccatc cagccctaat tcctatccat ttatctatgc adccat
tt cggccatcg ctttaggtgtt cccgcttccat gctatcttcc cagcagg
tt catccatccat cttccatccat ttatctatgc tccaaatccat tttatgc
tt acagegggttcc tccctcttcc tttatctatgc tccaaatccat tttatgc
cc tacgtattttcc ttctttccat tttatctatgc ctacaagaaaa acccttcc
ag ctcatgcataa ccacatccat ccccccggcc ctttcccccttccatccat



JD Eisenback

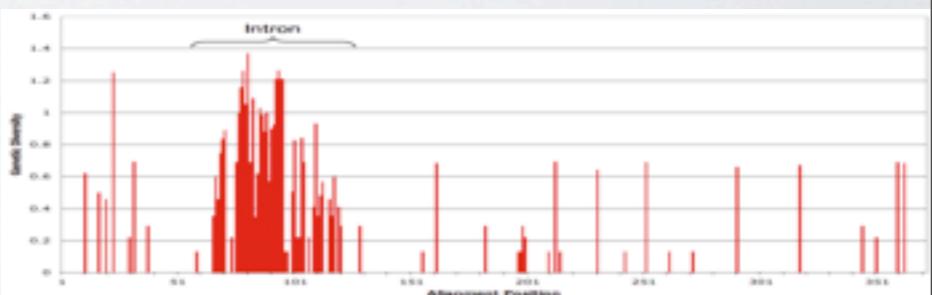
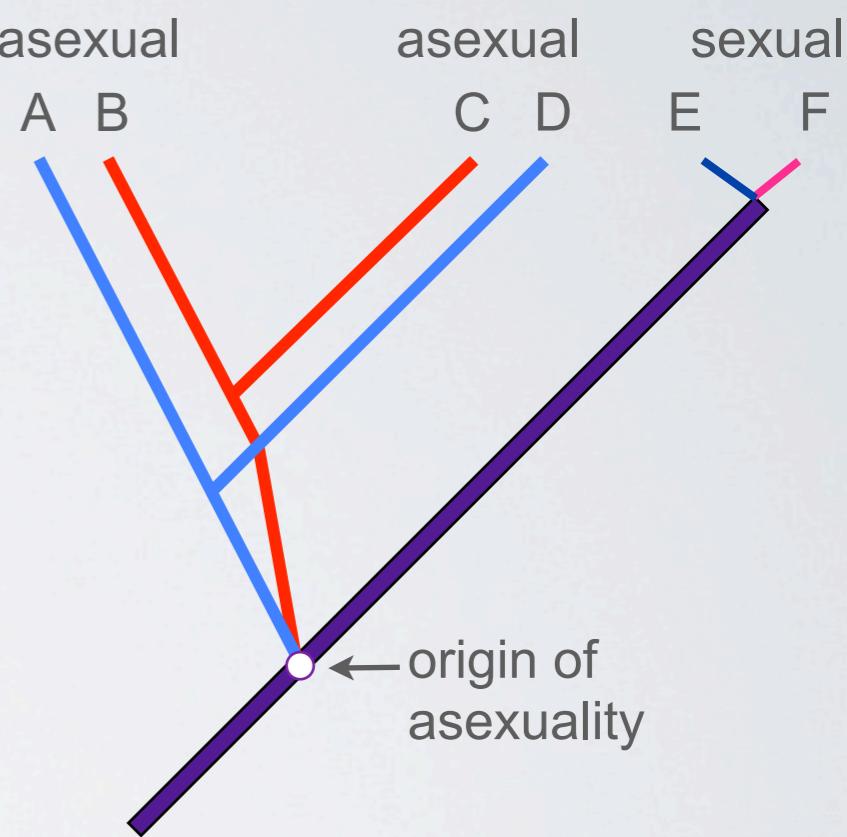


JD Eisenback

Recombination

- We can study recombination's action in genomic regions with reduced recombination- sex chromosomes, inversions
- We can also study its action in species that have lost meiotic recombination- asexuals
- **Mitotic reproduction has consequences for the genome-** decay of sex-specific genes, extreme 'Allelic' Sequence Divergence, loss of mutational effects of recombination

aa cccgcggcc tgatgtataa tttatgtt tacatggggattttcc
cc cccggatgta gtcgcacaca cttcccaaa tttttttttt cccaaq
cc ggaaacttcc ccctggaaact gaccatgaca ctaagccctt ttgaccc
ct tcatttcgt gatttcccat cattttataa gccttagctt tacatgt
ct acatcttttccatggatgtt gggatcggat tttgtatctc tacaagg
tc cgtttataa ataaacttcc ttatcccttataatgttggatggatgg
tt cttgccttcc taataatgtt ttatctacttataatgtttagttagt
tc ttcaacttca cacacatgtt ttctcttataatgttggatggatgg
ca actatcccttacggatcatacg aaatccatccatccatgcac taggtcc
ag ggccatcata cggcccttatc ttatccatccatccatgcac taggtcc
tt cggccatccatccatgcac taggtccatccatccatgcac taggtcc
tt catcaatttccatccatgcac taggtccatccatgcac taggtcc
tt acatccatccatgcac taggtccatccatgcac taggtccatccatgc
cc tacgtattttccatccatgcac taggtccatccatgcac taggtccatcc
og ctcatgcata ccacatgcata gccccagcc cttggccccc accatcc
ccatccatccatgcata ccacatgcata gccccagcc cttggccccc accatcc



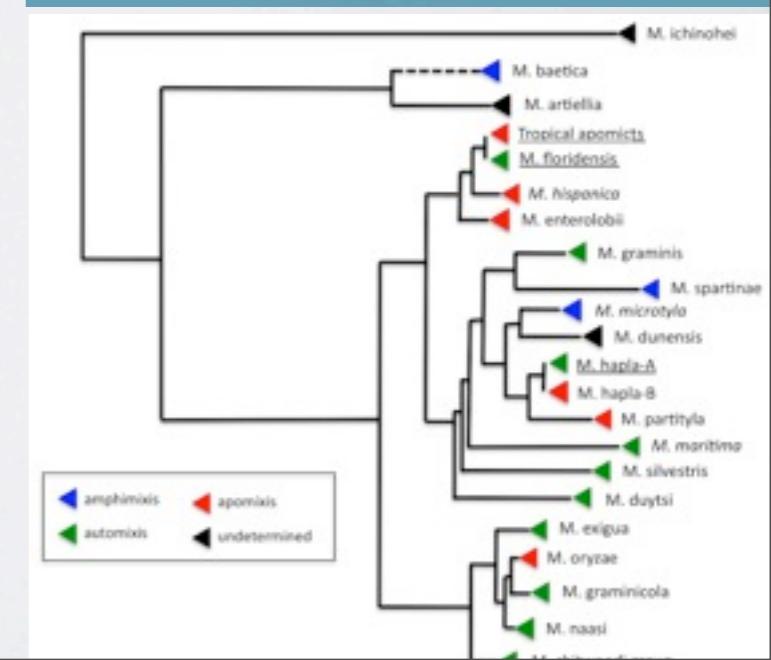
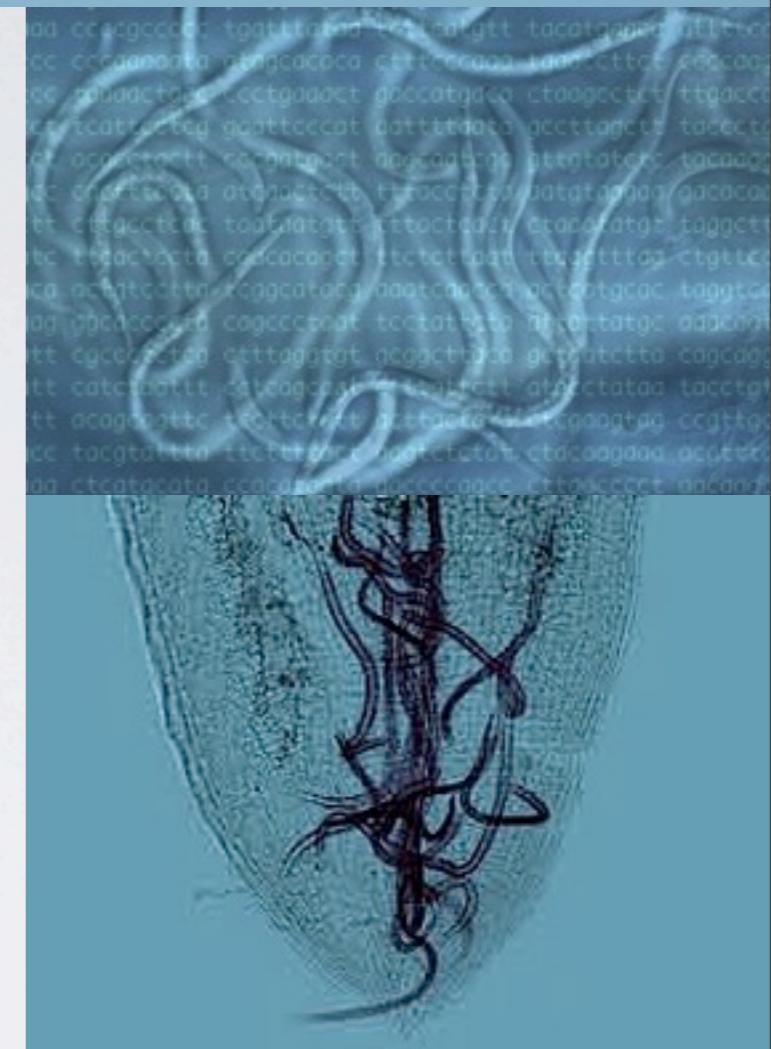
Meloidogyne Root Knot Nematodes

- Globally important agricultural pest species
- Enormous plant host range
 - parasitize all main crop plants
- ~5% loss of world agriculture



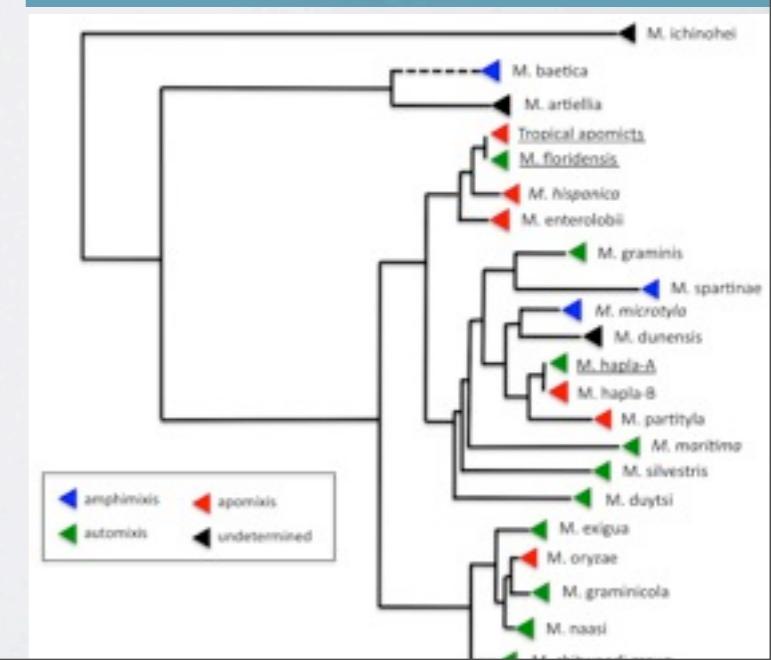
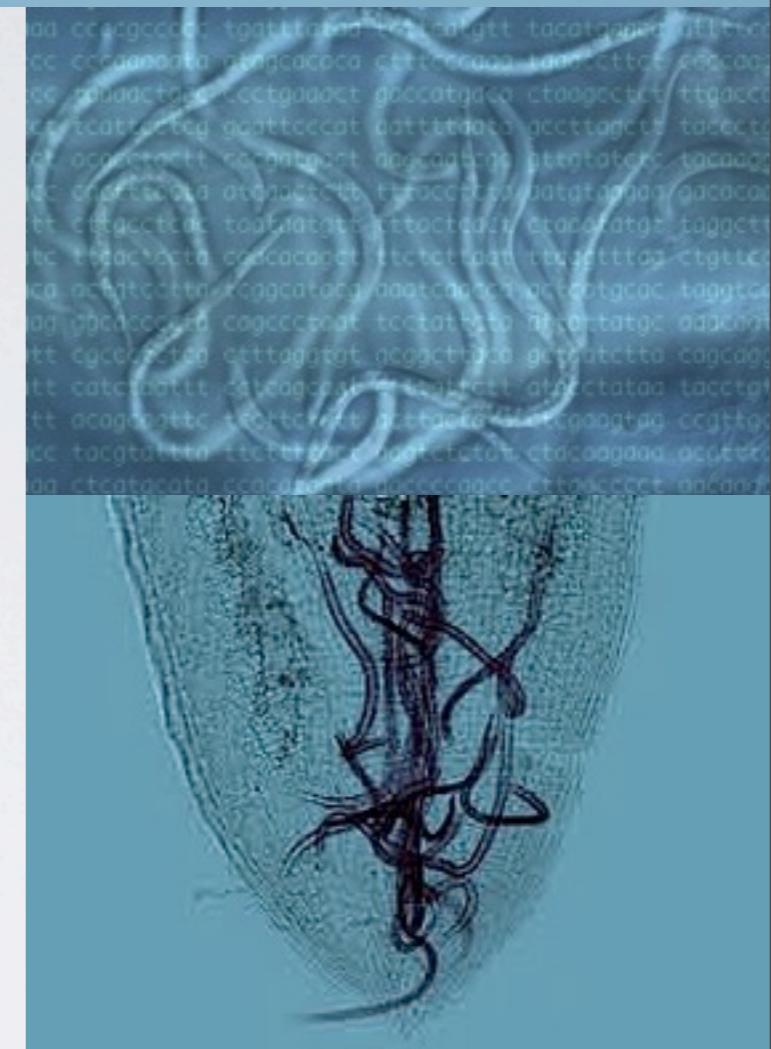
Meloidogyne Reproduction

- Wide variety of reproductive modes in a single genus
- Many species are mitotic parthenogens without chromosome pairs
 - Incapable of meiosis
 - Could be ‘ancient’ asexuals
 - 17 million years without meiosis?



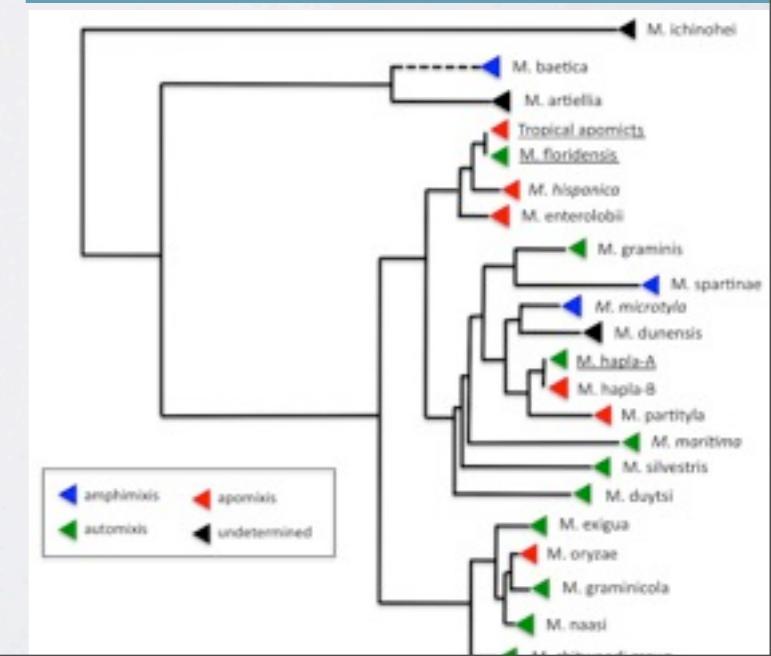
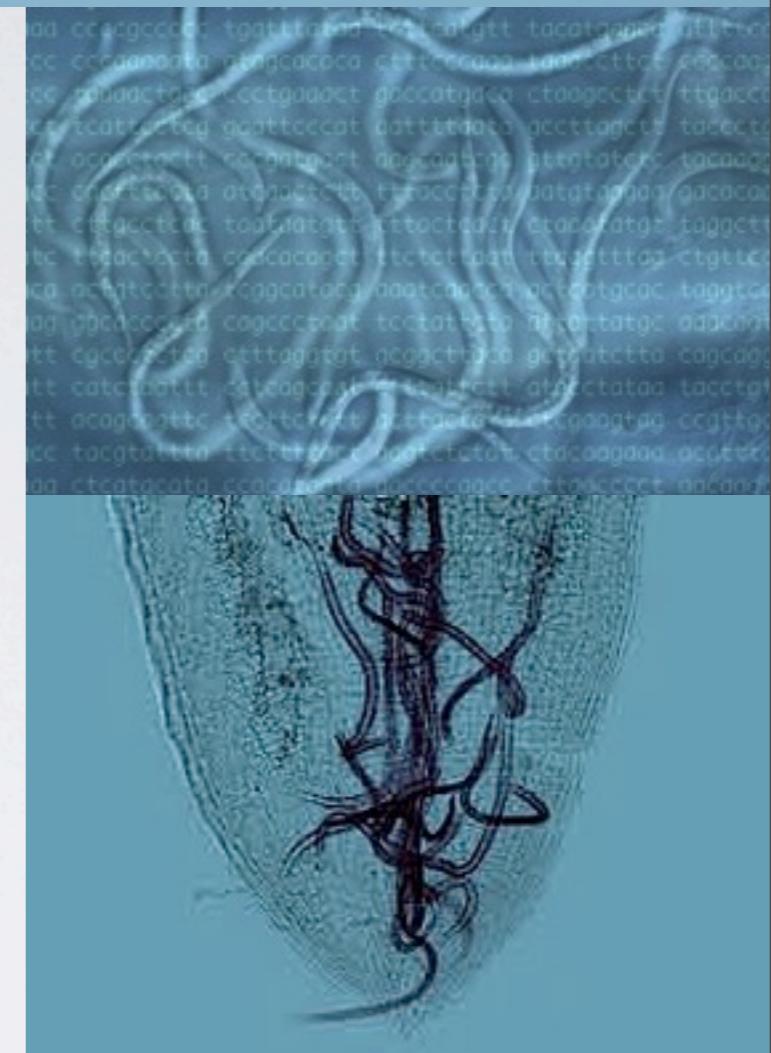
Meloidogyne Reproduction

- Wide variety of reproductive modes in a single genus
- Many species are mitotic parthenogens without chromosome pairs
- Other species are meiotic parthenogens
 - automixis



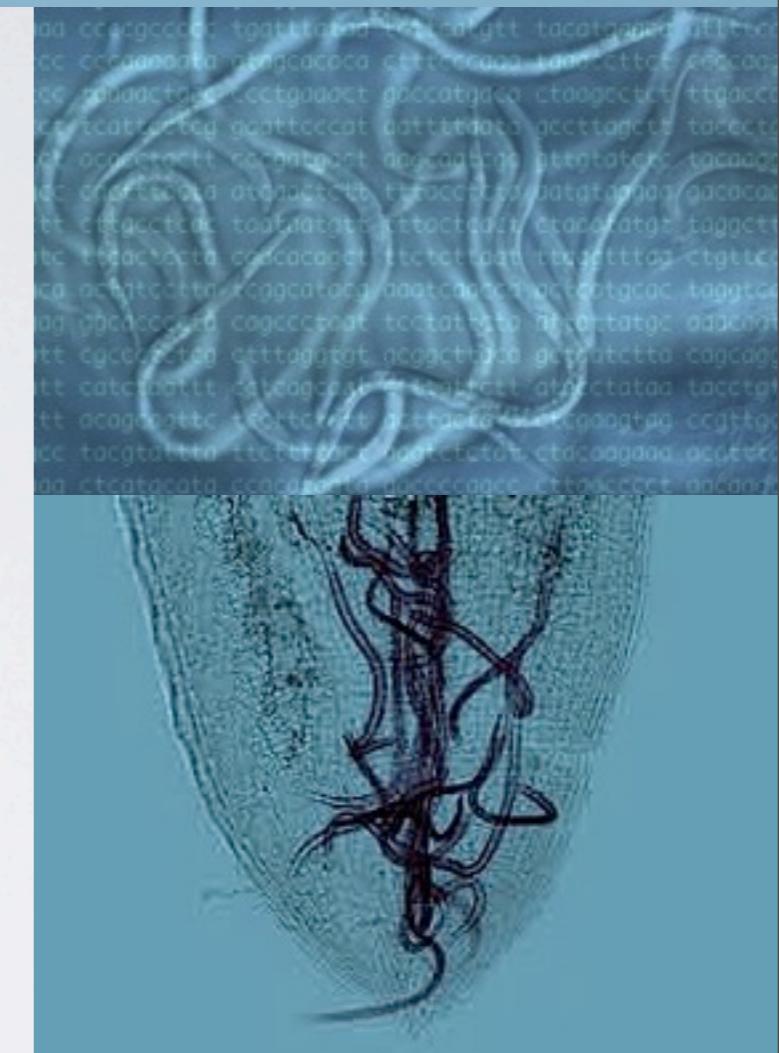
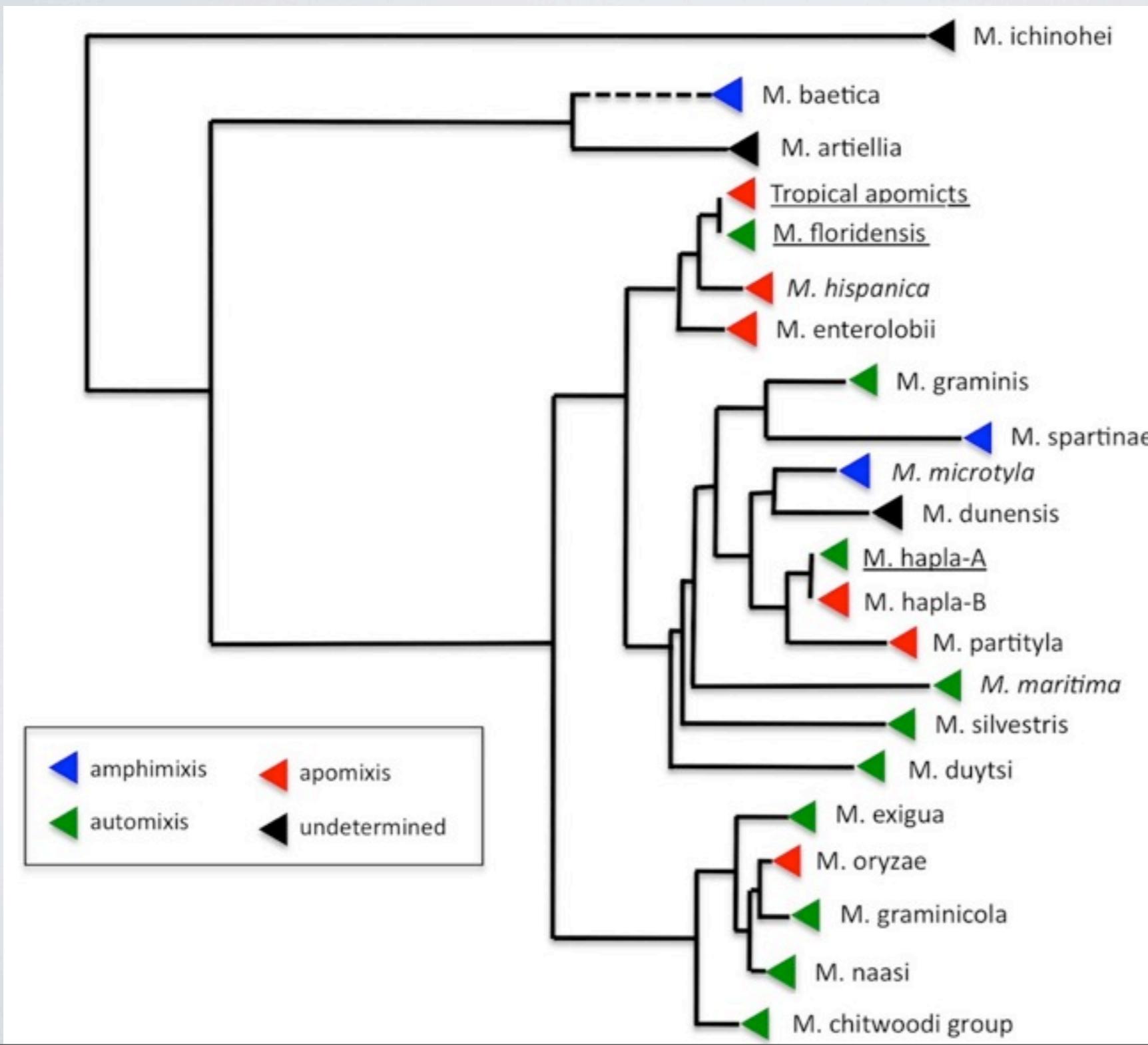
Meloidogyne Reproduction

- Wide variety of reproductive modes in a single genus
- Many species are mitotic parthenogens without chromosome pairs
- Other species are meiotic parthenogens
- Some species are obligatory outbreeding sexuals with males & females
 - amphimixis



Meloidogyne Reproduction

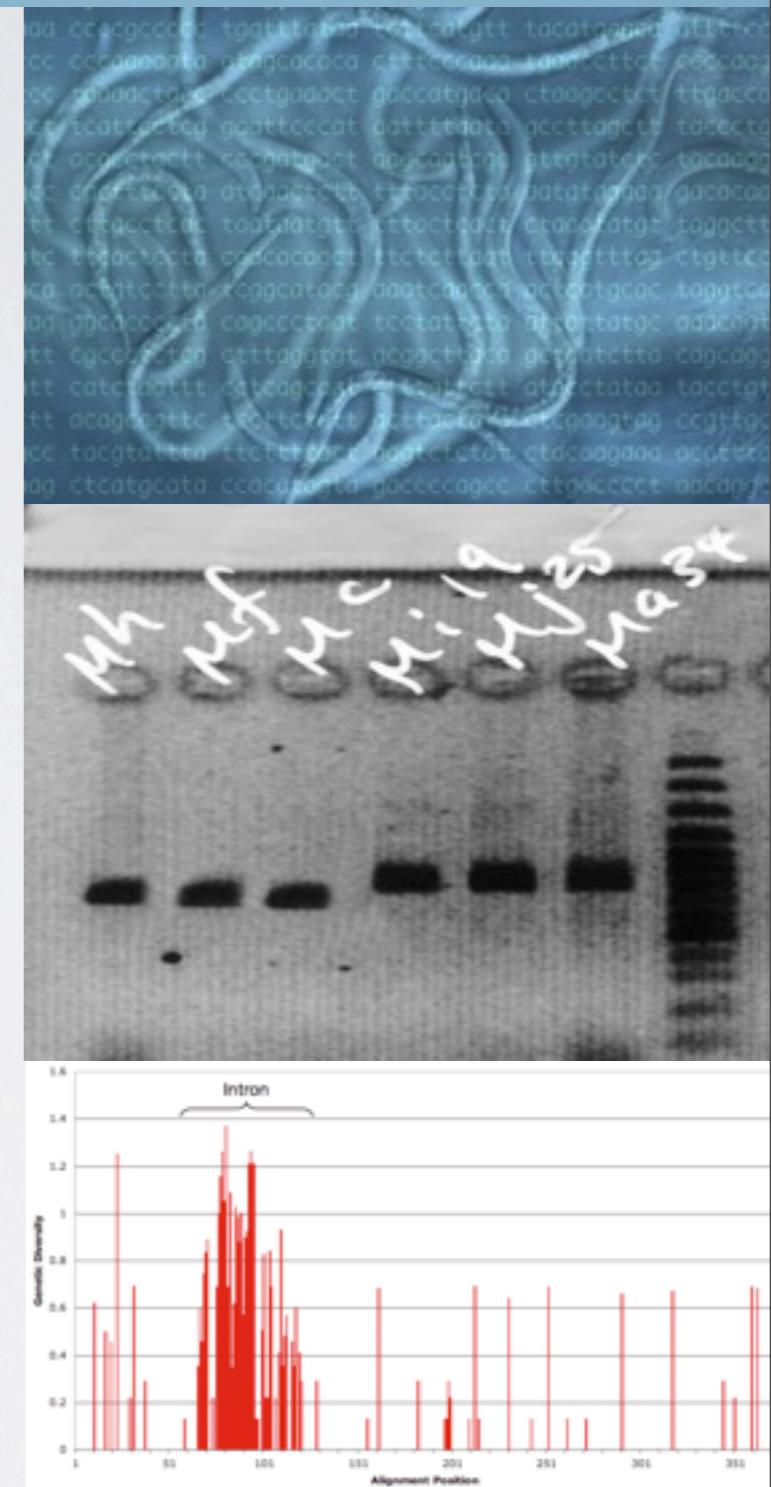
- Wide variety of reproductive modes in a single genus



Previous Single Gene Sequencing

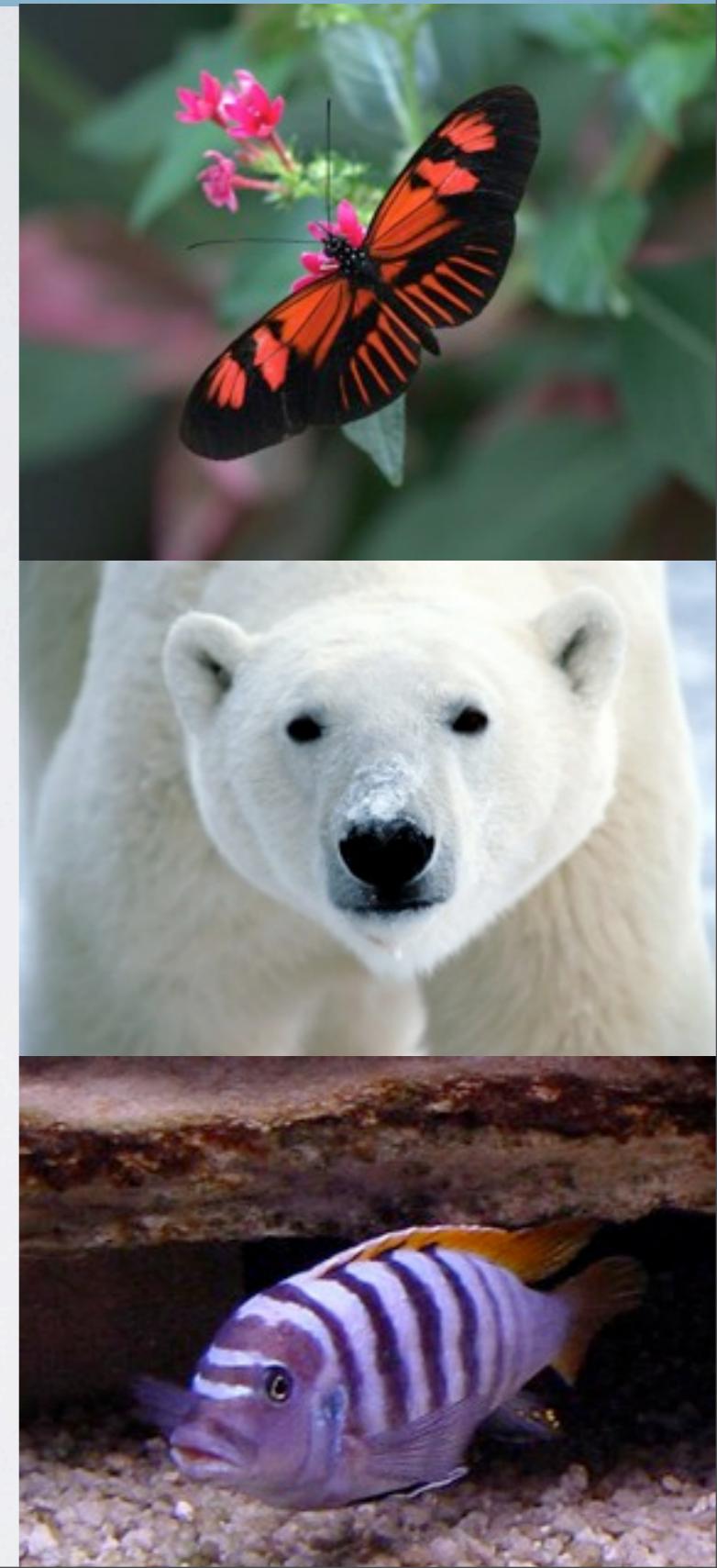
Lunt DH 2008 BMC Evolutionary Biology 8:194

- I can reject *ancient asexuality* on basis of interspecific allele sharing and identical molecular evolution of sperm protein genes
- Data suggests interspecific hybrid origins



Hybrid Speciation

- Once thought that hybrid speciation was rare and inconsequential in animals
- Genome biology is revealing a very different view
- We have investigated the origins of *Meloidogyne* asexuals in this context
 - Hybrid speciation? Investigate origins of asexuals with whole genome sequences in a phylogenetic design

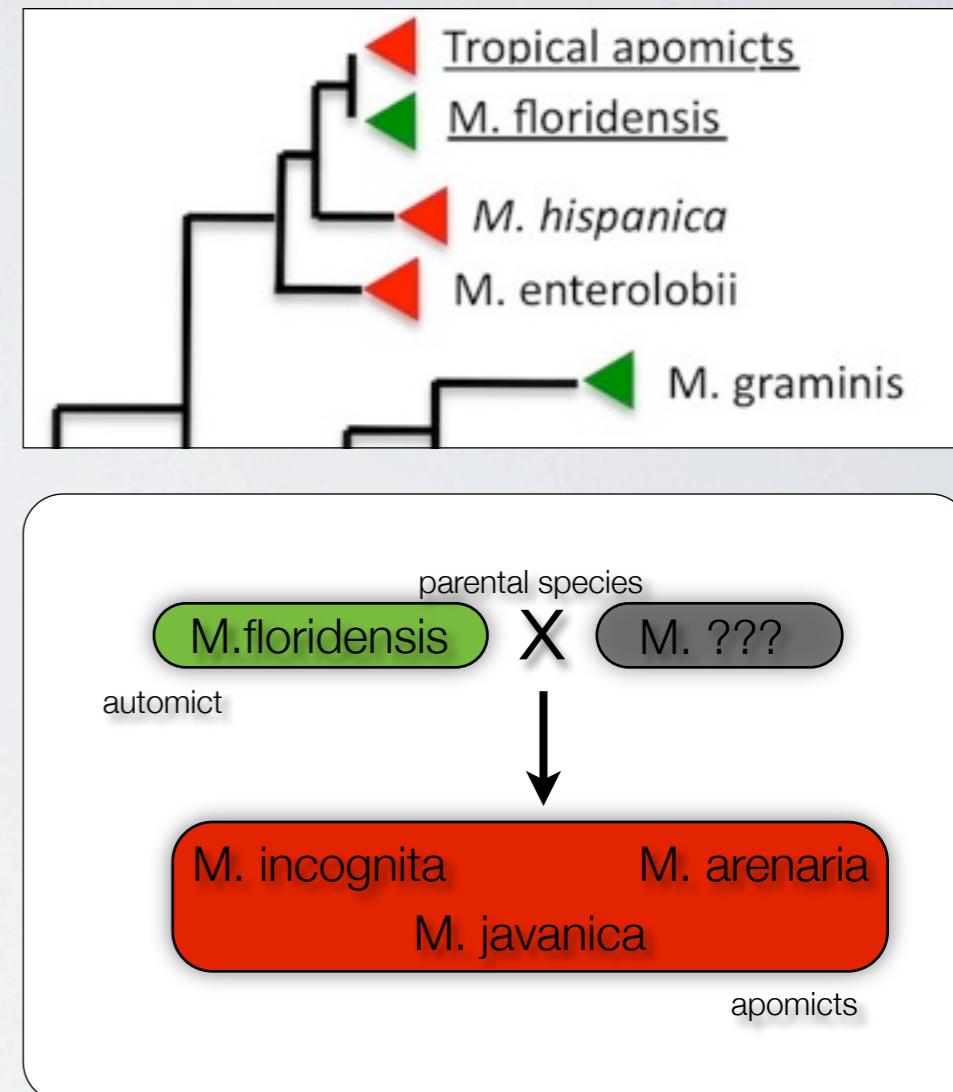


Is *M. floridensis* the parent of the asexuals?

Investigated using whole genome sequences;

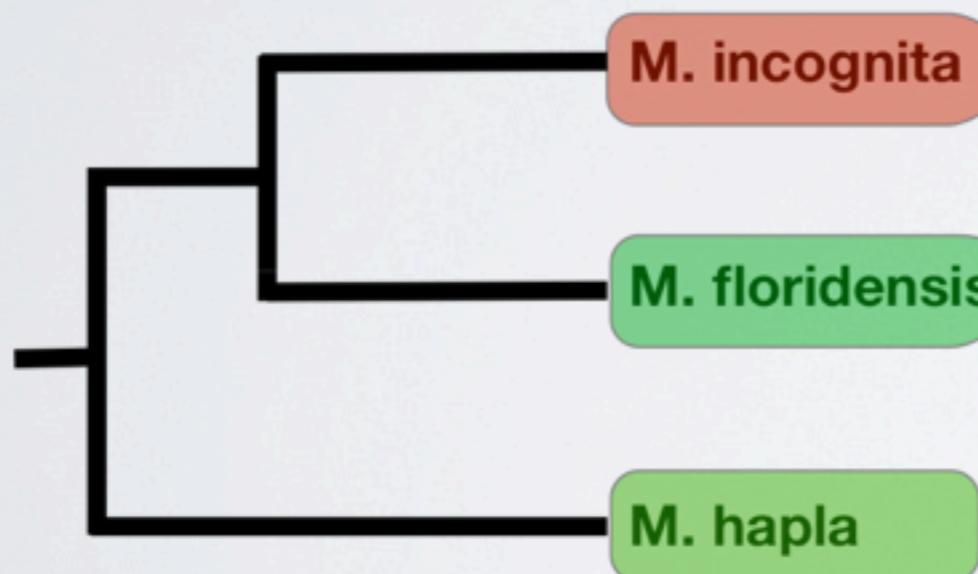
--look at the within-genome patterns of diversity to determine hybrid nature of genomes

--look at phylogenetic relationships of all genes to study origins and parents



Meloidogyne comparative genomics

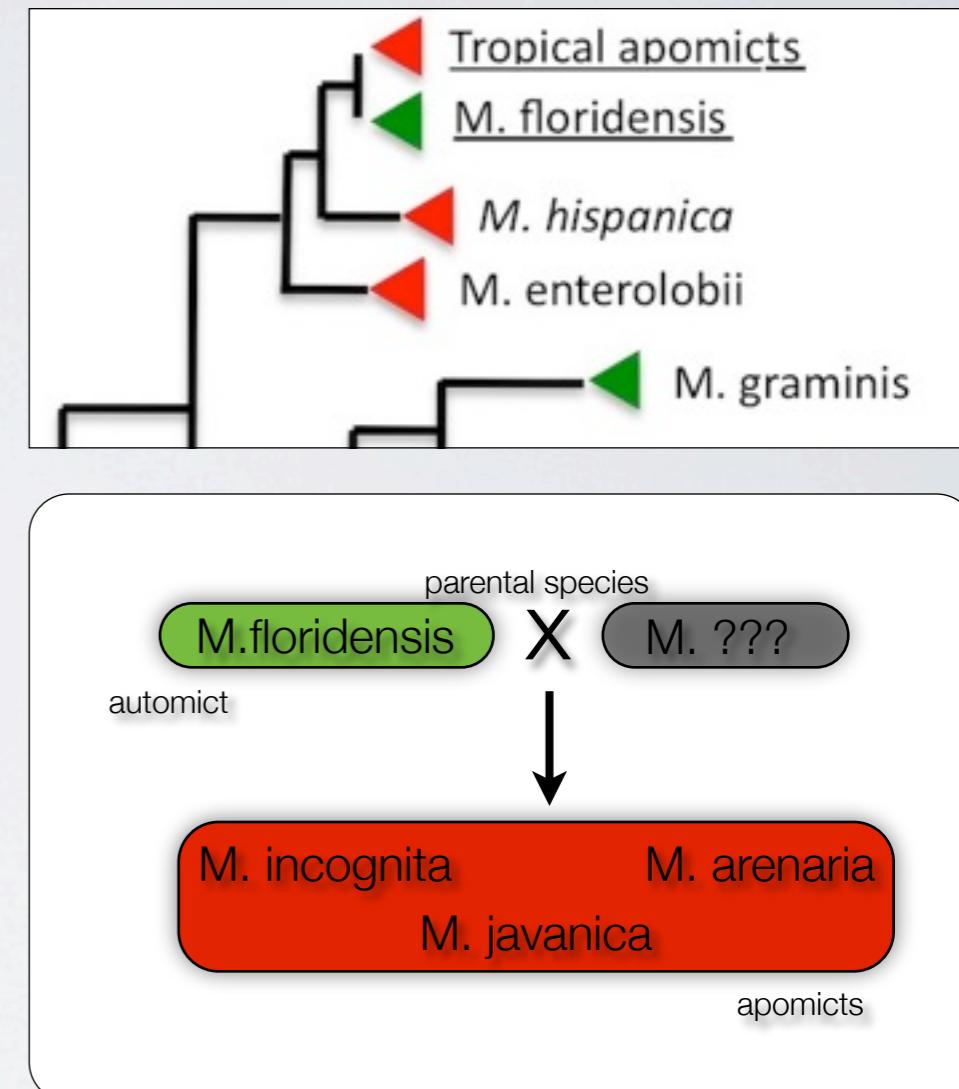
We have sequenced *M. floridensis* genome and are able to compare to 2 other *Meloidogyne* genomes published by other groups



asexual, hybrid?

sexual, parental?

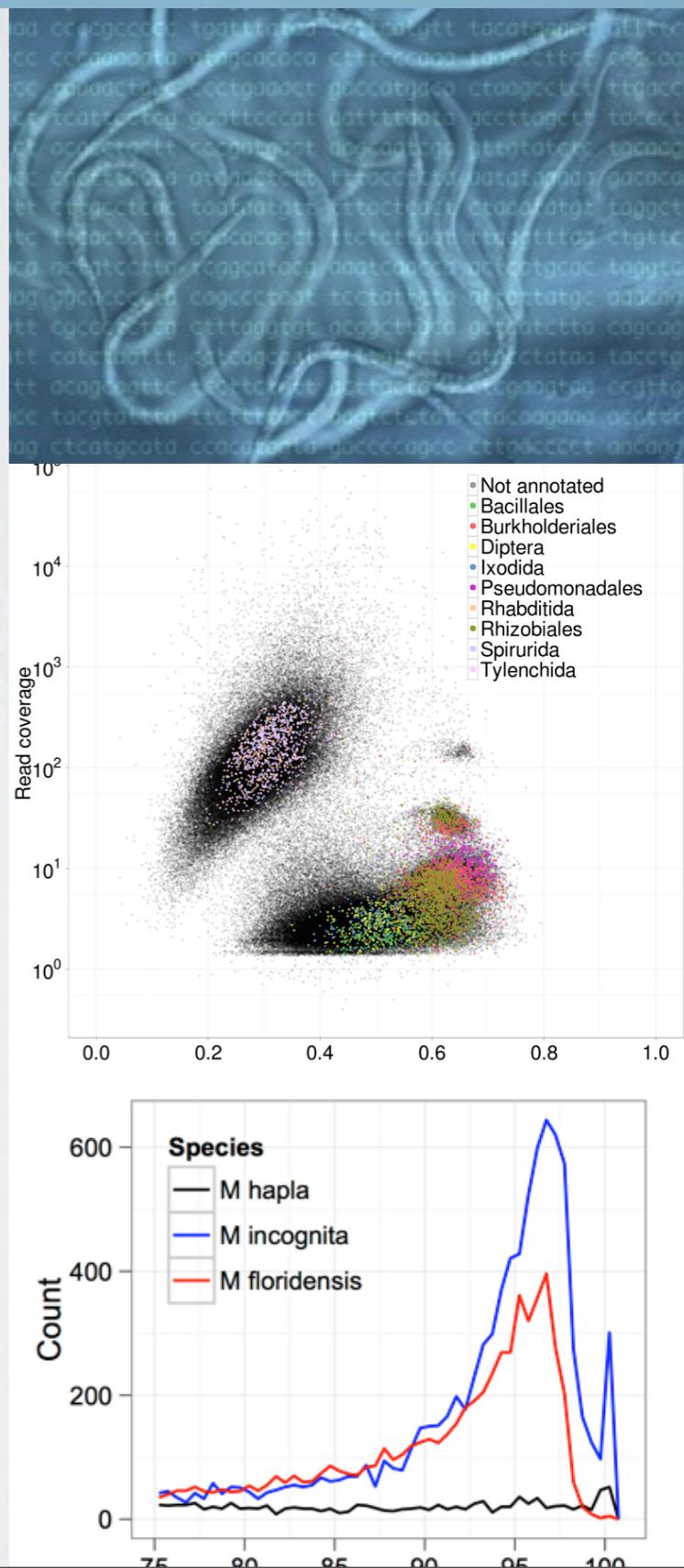
sexual, outgroup



The *Meloidogyne floridensis* genome

- Illumina HiSeq2000 v2 reagents
 - 100bp paired end
 - 250bp fragments
 - 81k scaffolds
 - N50 3.5k
 - 30% GC

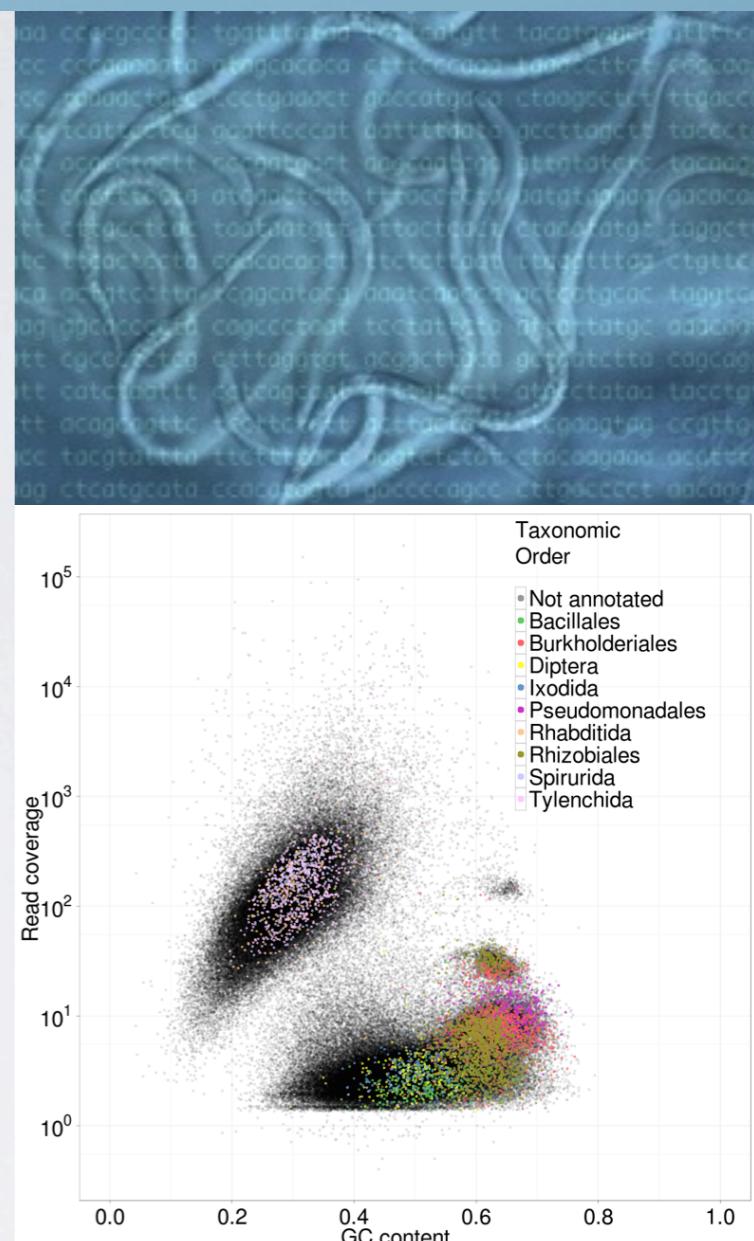
M. floridensis draft genome raw data SRA ERP001338



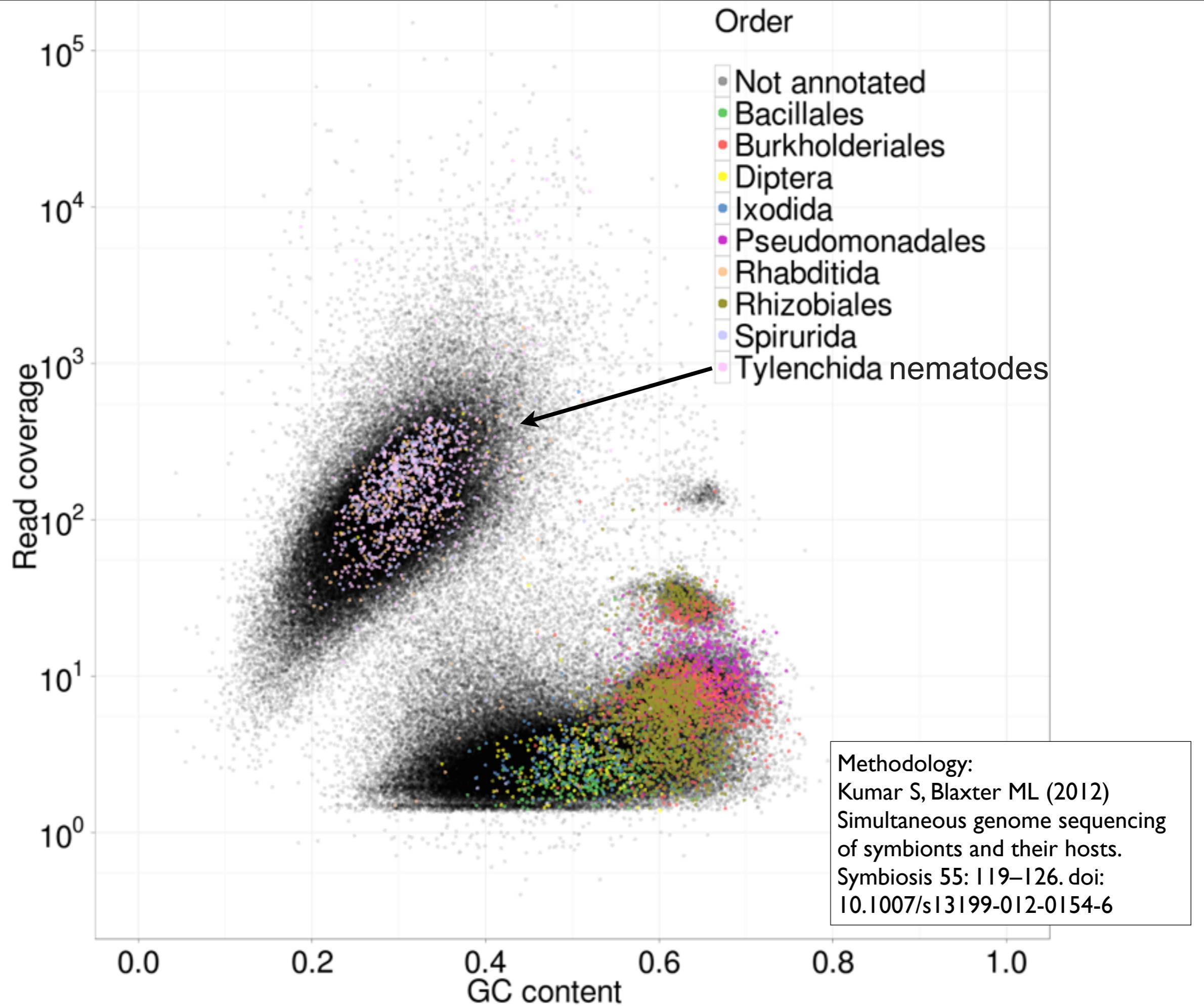
Cleaning the *M. floridensis* genome

DNA isolated from nematodes on plant roots will include many microbial ‘contaminants’

- Preliminary assembly of trimmed reads ignoring pairing information
- Determine %GC and read coverage
- Annotate contigs with taxonomic info determined by megablast
- Scatterplot

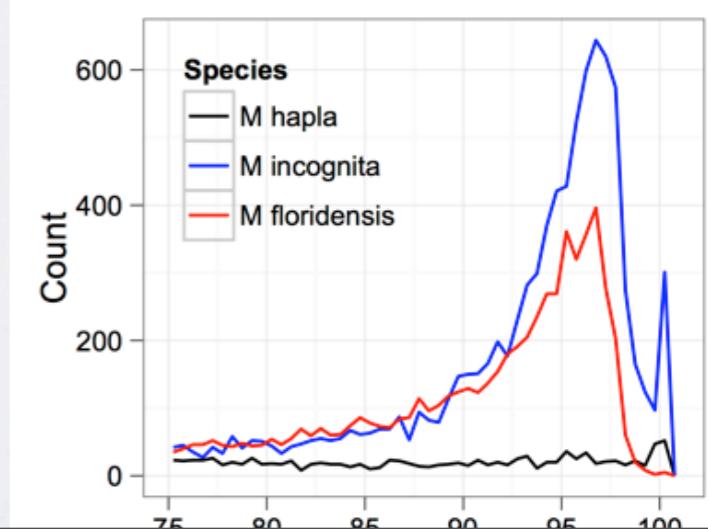
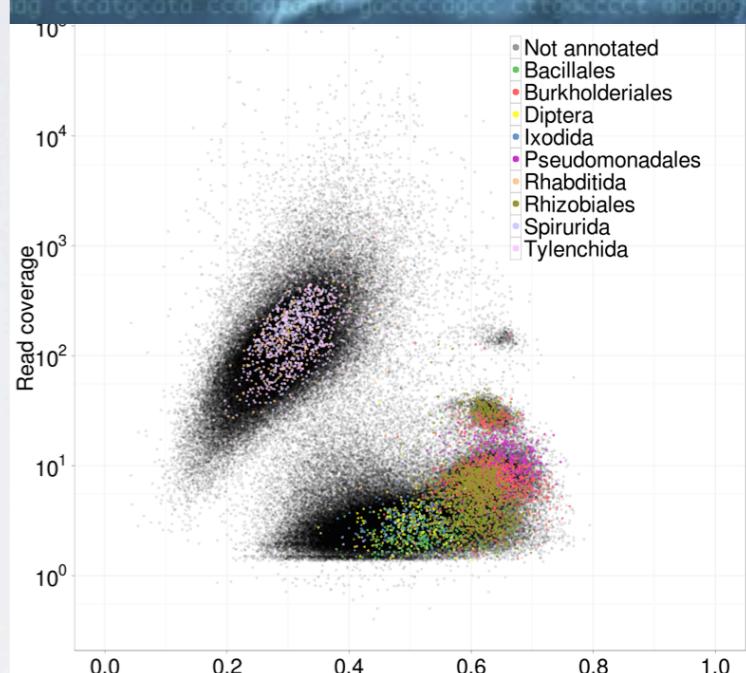


Kumar S, Blaxter ML (2012)
Simultaneous genome sequencing of symbionts and their hosts.
Symbiosis 55: 119–126. doi:10.1007/s13199-012-0154-6



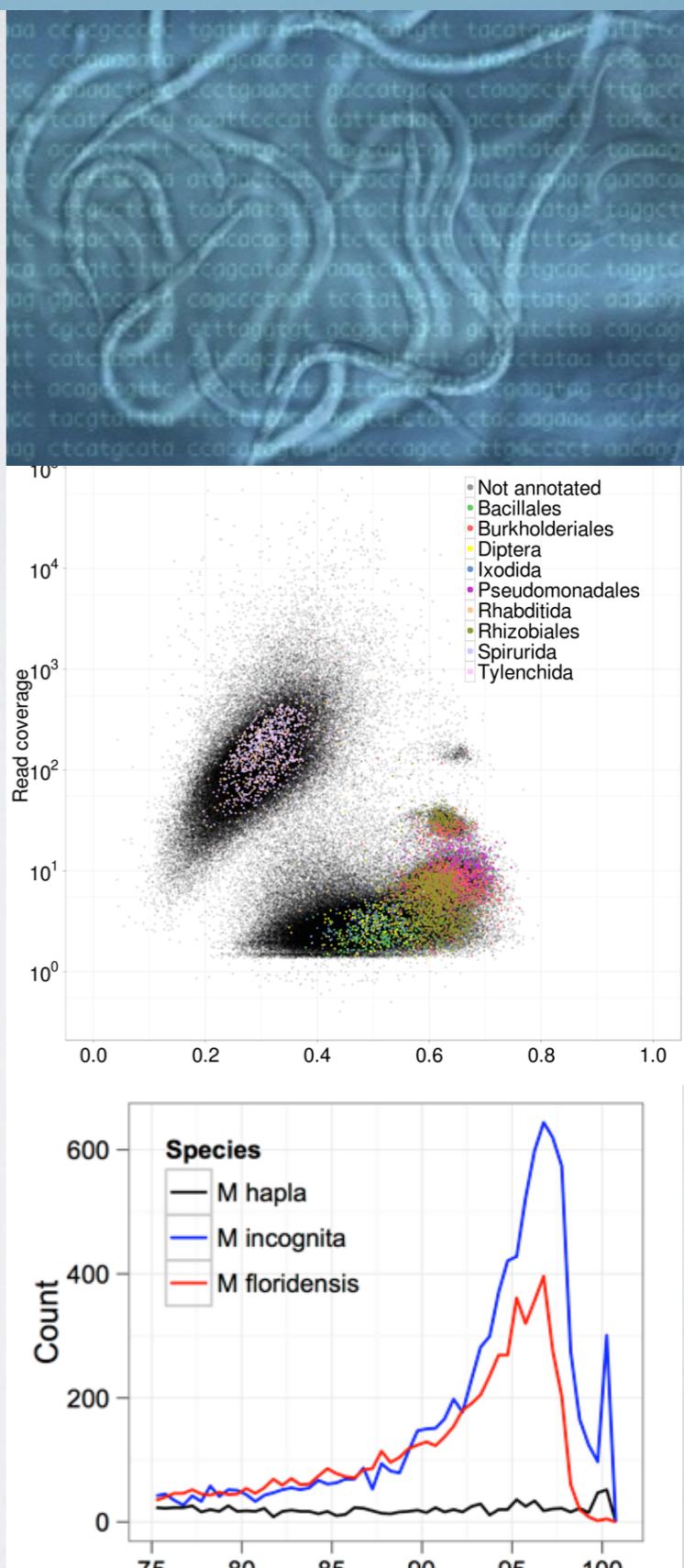
The *Meloidogyne floridensis* genome

- 100Mb assembly ~100x genomic coverage
- 15.3k predicted proteins
- Directly comparable to published *Meloidogyne* genomes



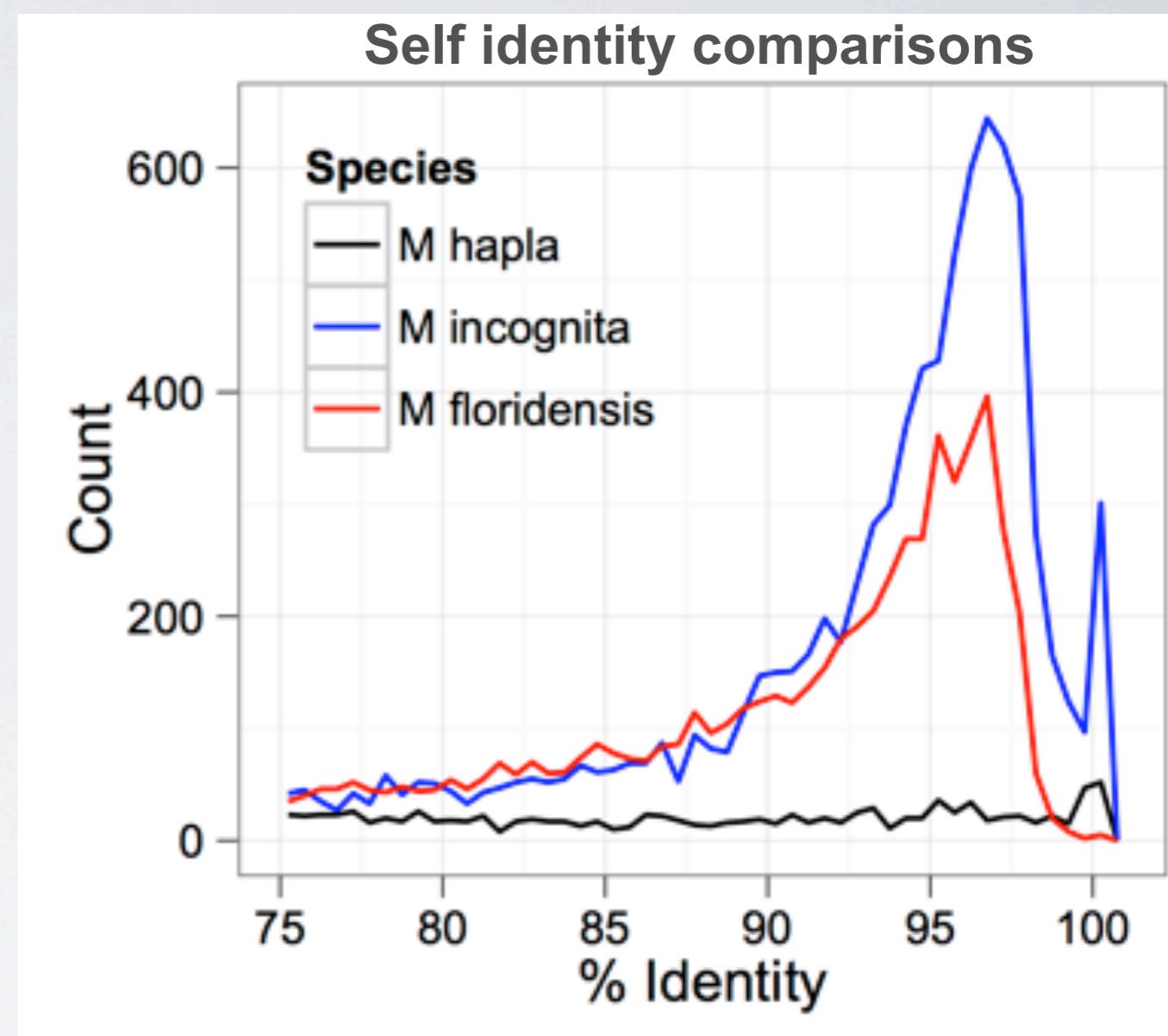
Comparative genomics questions

- Is there evidence of hybrid origins of asexual species?
 - Is *M. floridensis* a parental?
 - How do offspring and parental genomes differ?
 - Broader implications?



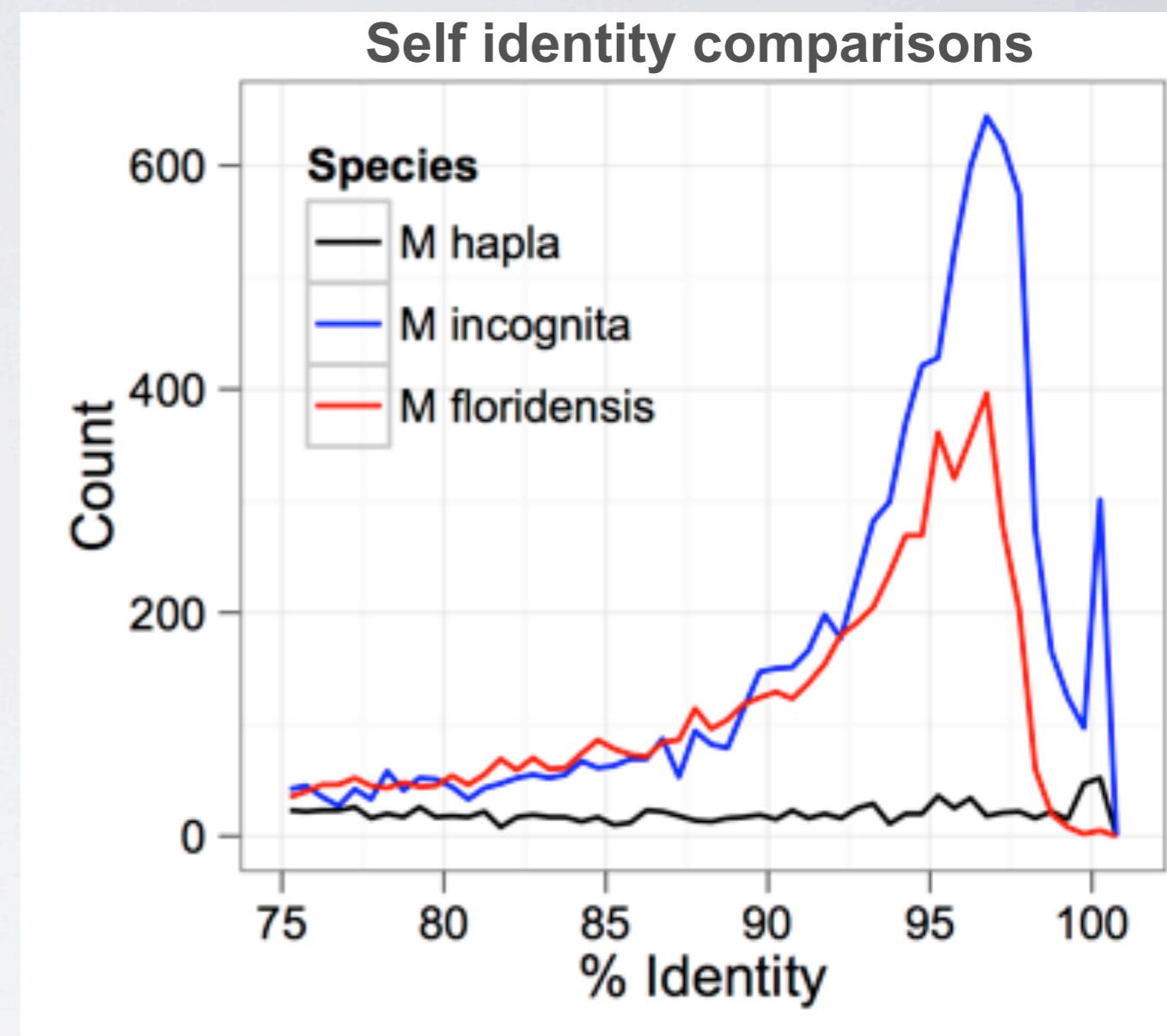
ID of duplicated protein-coding regions

- Coding sequences from each of the three target genomes (*M. hapla*, *M. incognita* and *M. floridensis*) were compared to the set of genes from the same species
- The percent identity of the best matching (non-self) coding sequence was calculated, and is plotted as a frequency histogram
- Both *M. incognita* and *M. floridensis* show evidence of presence of many duplicates, while *M. hapla* does not



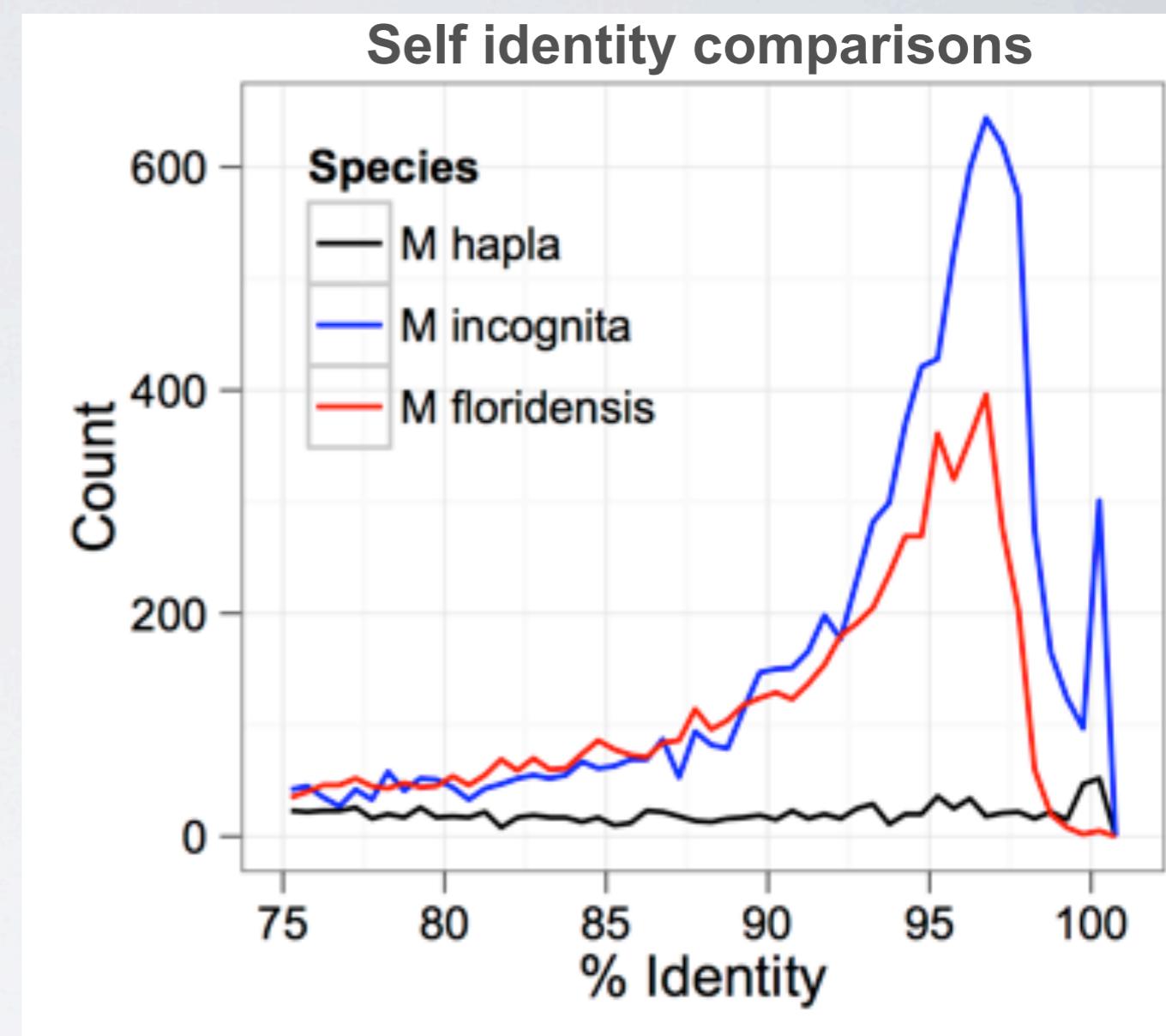
ID of duplicated protein-coding regions

- Coding sequences from each of the three target genomes (*M. hapla*, *M. incognita* and *M. floridensis*) were compared to the set of genes from the same species
- The percent identity of the best matching (non-self) coding sequence was calculated, and is plotted as a frequency histogram
- Both *M. incognita* and *M. floridensis* show evidence of presence of many duplicates, while *M. hapla* does not



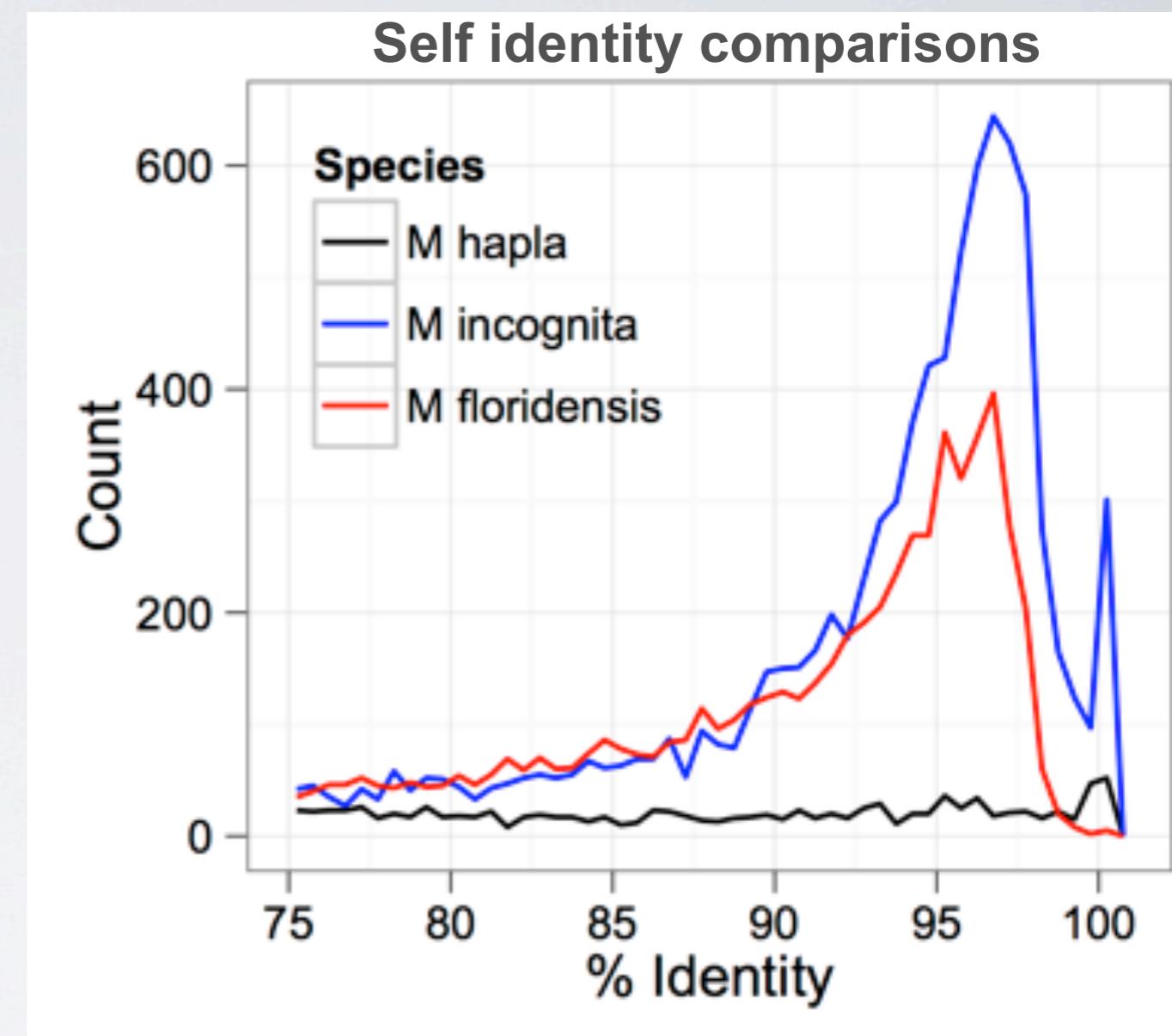
ID of duplicated protein-coding regions

- Coding sequences from each of the three target genomes (*M. hapla*, *M. incognita* and *M. floridensis*) were compared to the set of genes from the same species
- The percent identity of the best matching (non-self) coding sequence was calculated, and is plotted as a frequency histogram
- Both *M. incognita* and *M. floridensis* show evidence of presence of many duplicates, while *M. hapla* does not



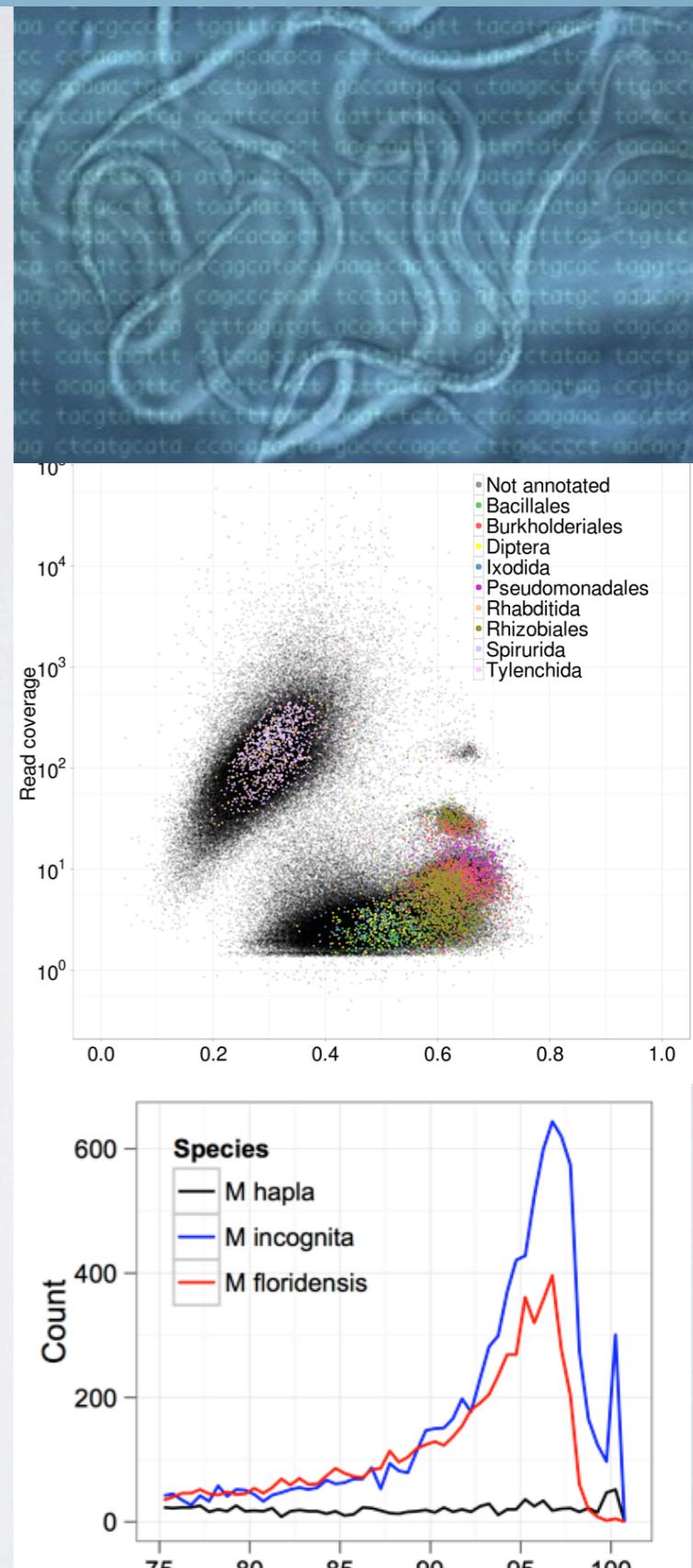
ID of duplicated protein-coding regions

- We have strong evidence that both *M. incognita* and *M. floridensis* contain diverged gene copies.
 - These loci duplicated at approximately the same point in time.
 - A ploidy change is not involved.
- This is expected pattern for hybrid genomes



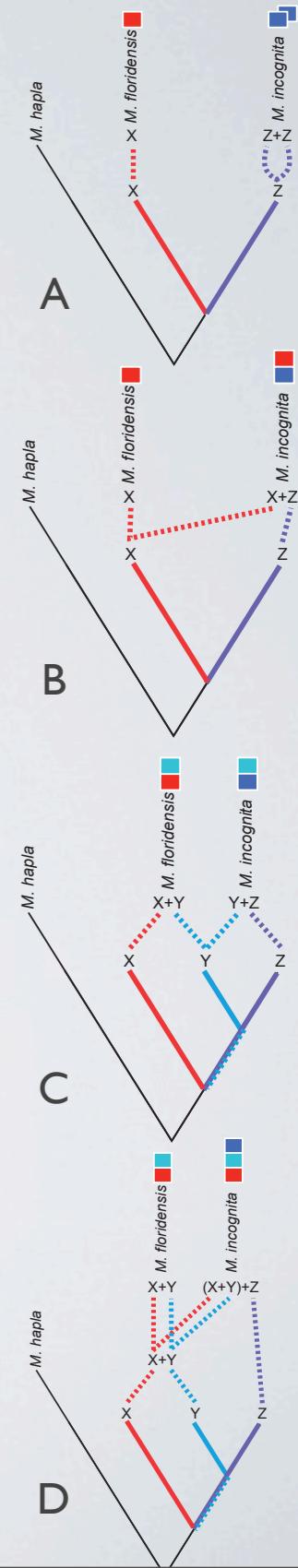
M. floridensis Genome Size

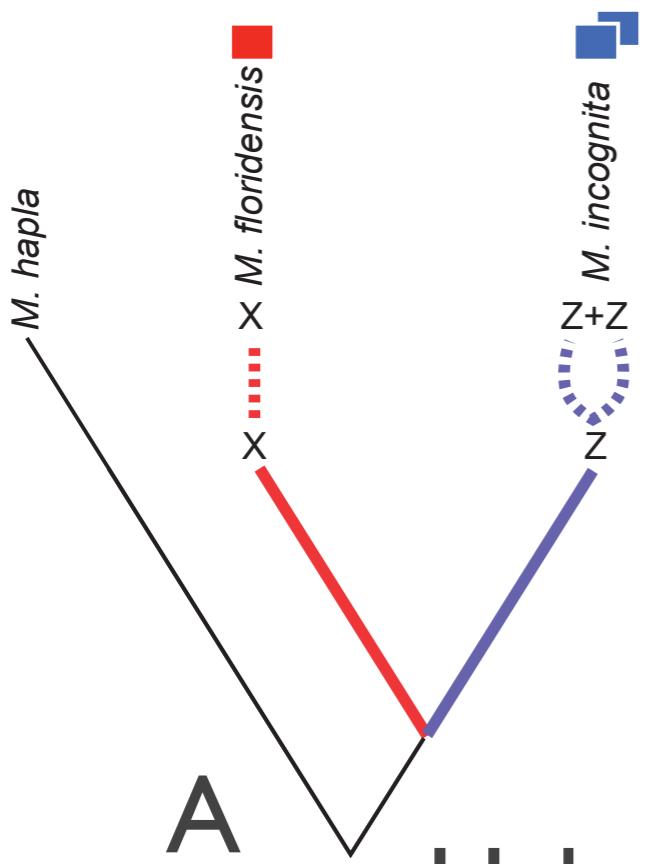
- Assembly size is not haploid genome size for hybrid species
- Divergence (4-8%) between homeologous (hybrid) copies will preclude assembly
- Our assembly of 100Mb is ~2x 50-54Mb genome size of *M. hapla*



Hybridization Hypotheses

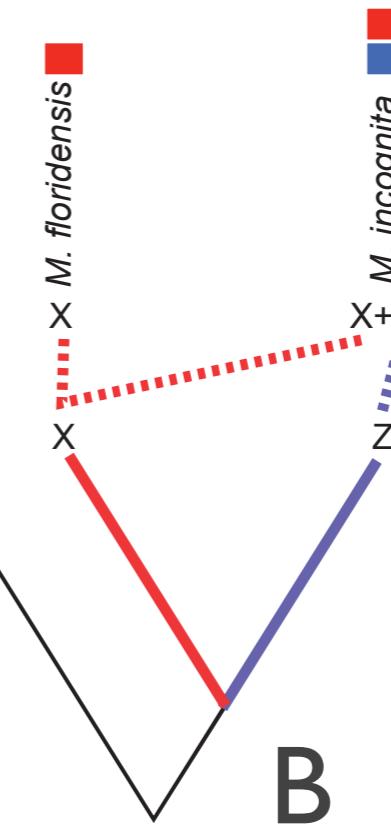
- There are very many ways species could hybridize, duplicate genes, lose genes
- We have selected a broad range of possibilities informed by prior knowledge
- We have tested their predictions phylogenetically



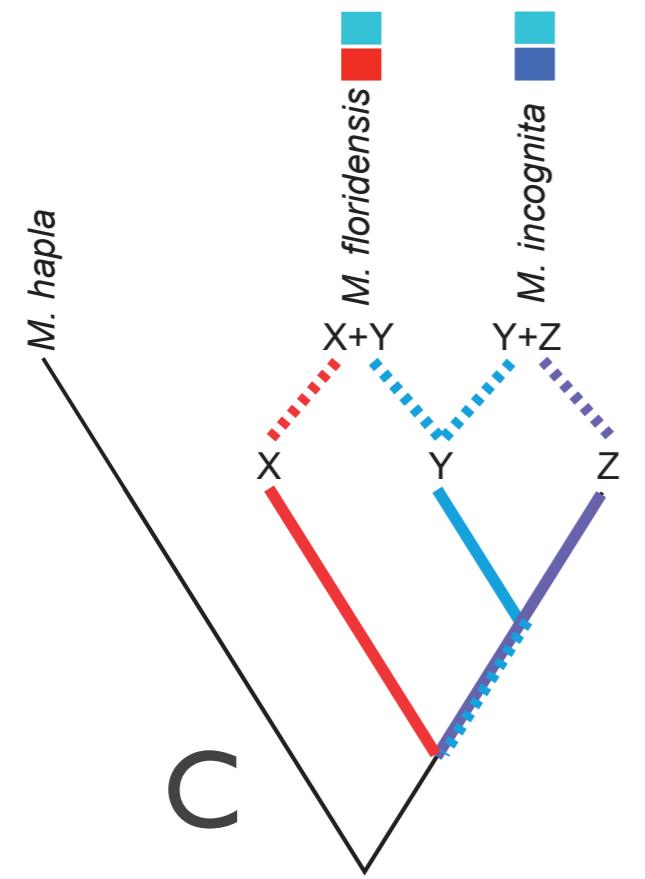


A

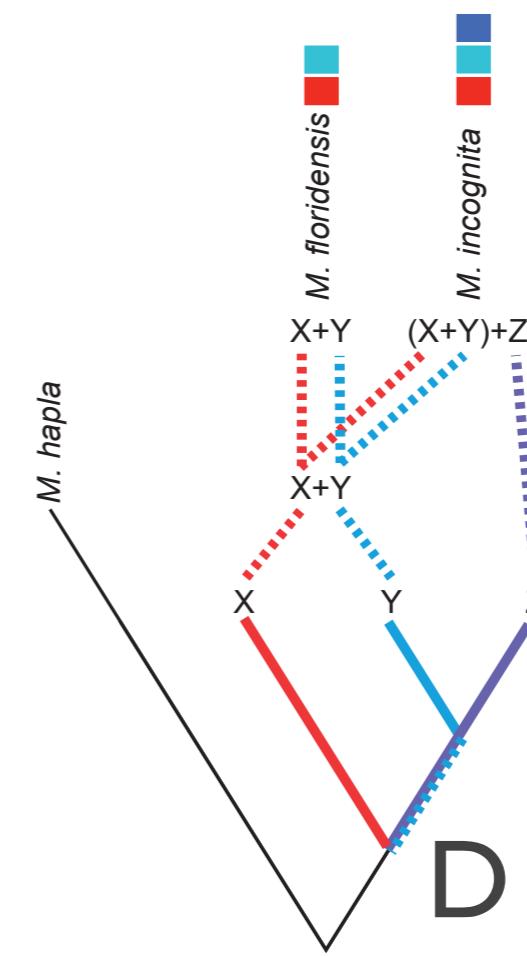
Hybridization hypotheses



B



C



D

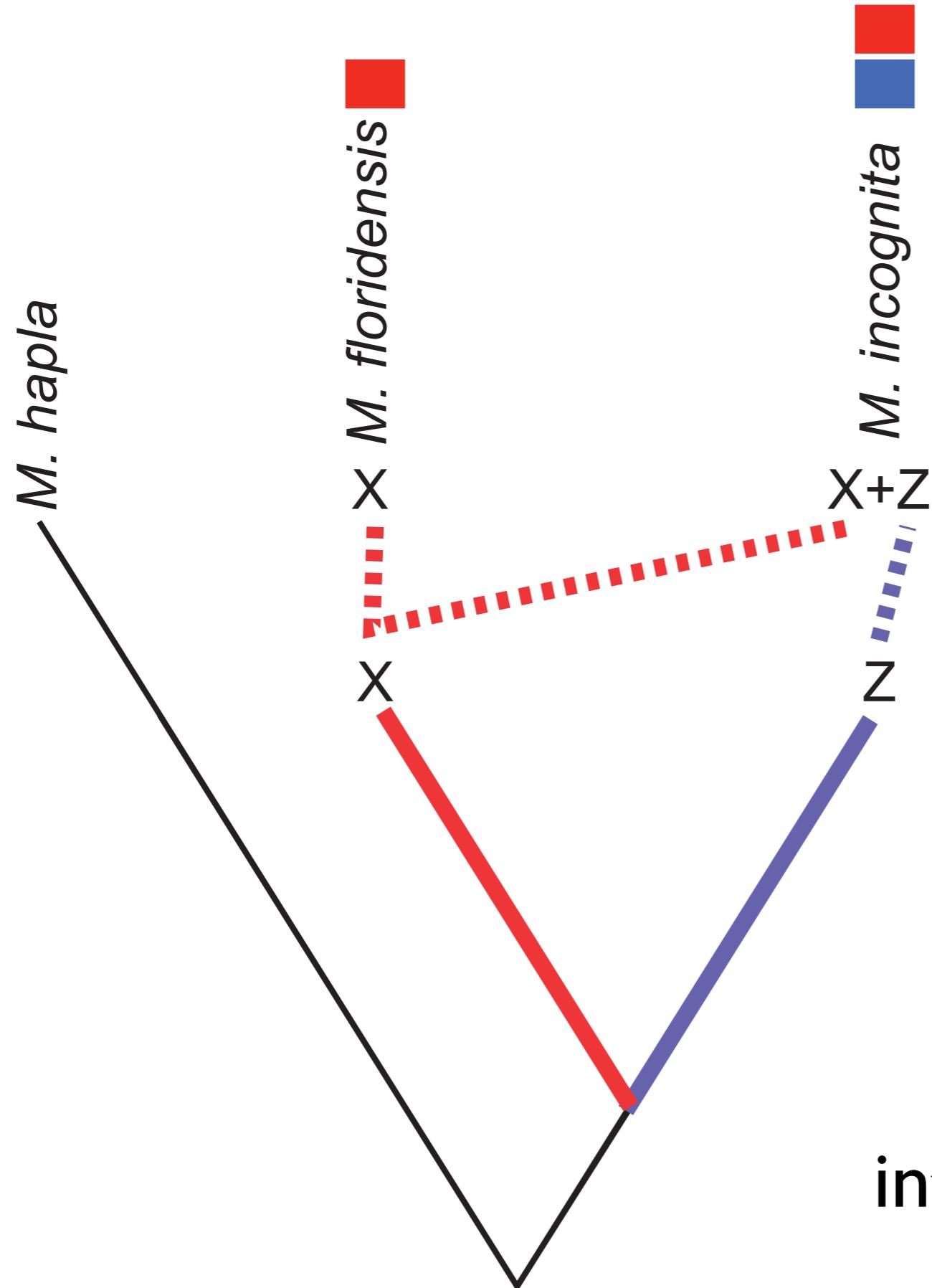
- *M. hapla*

\times *M. floridensis*

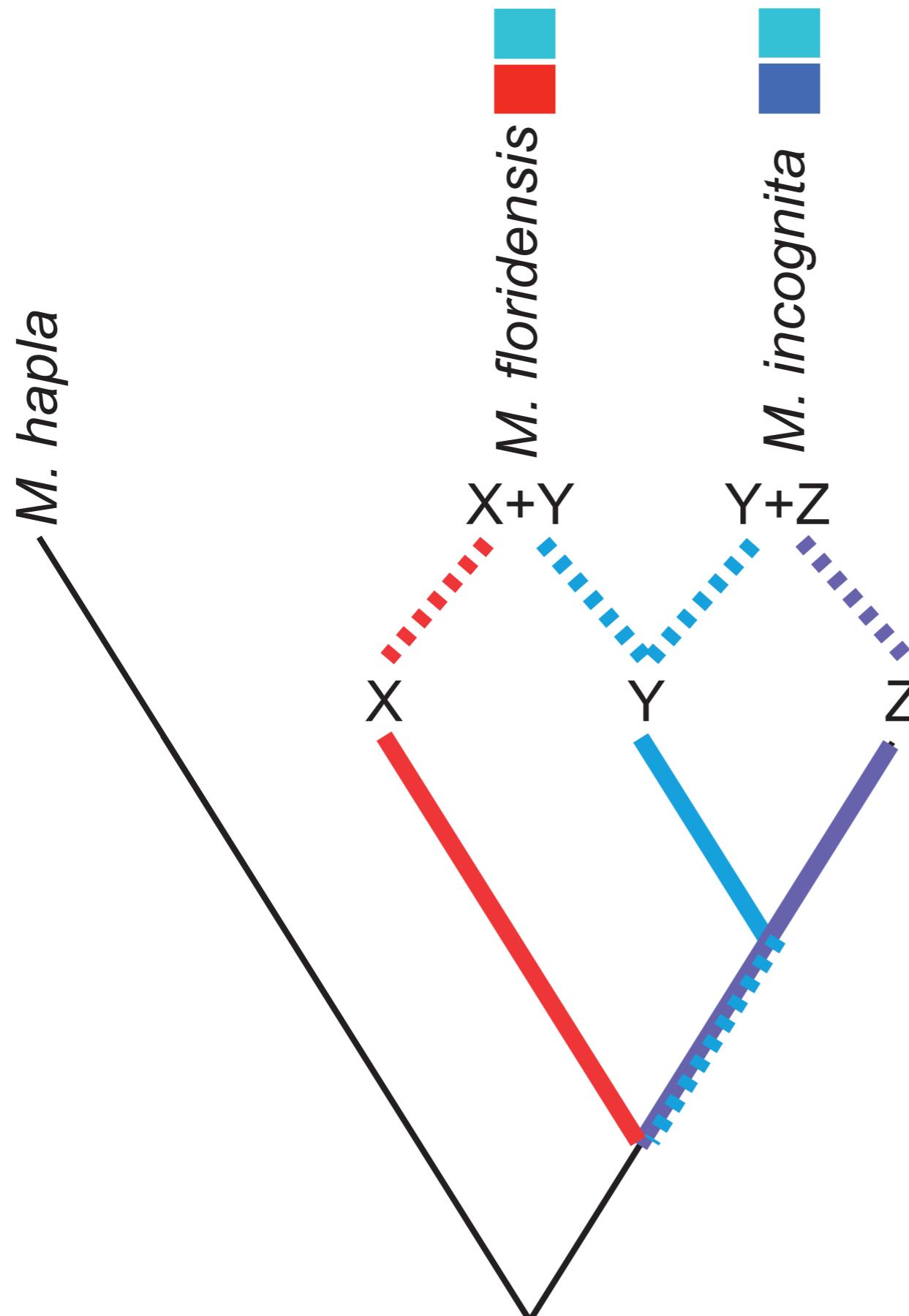
N + N *M. incognita*

(A)

Whole genome duplication(s)

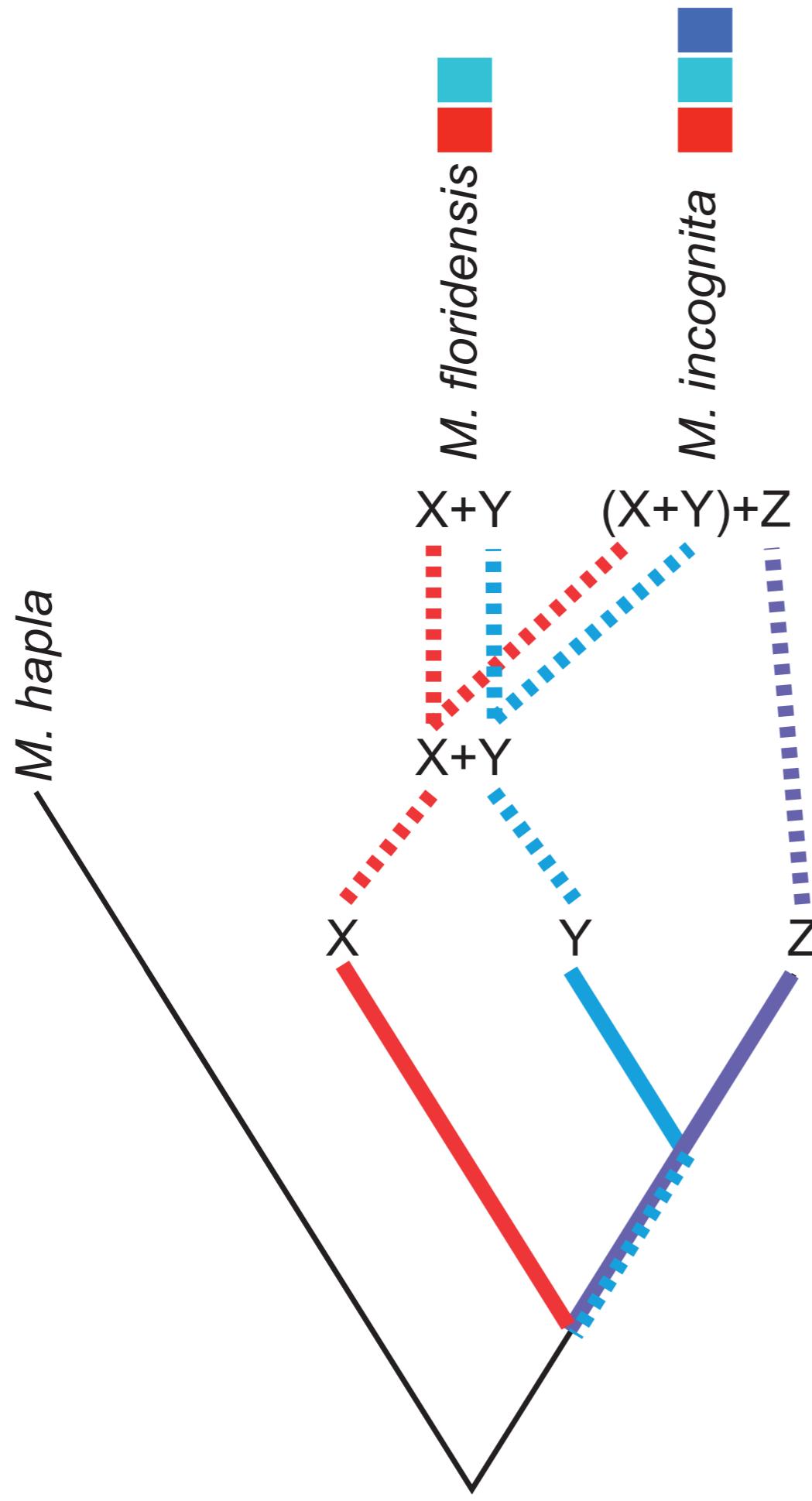


(B)
M. incognita is an
interspecific hybrid with
M. floridensis as one
parent



(C)

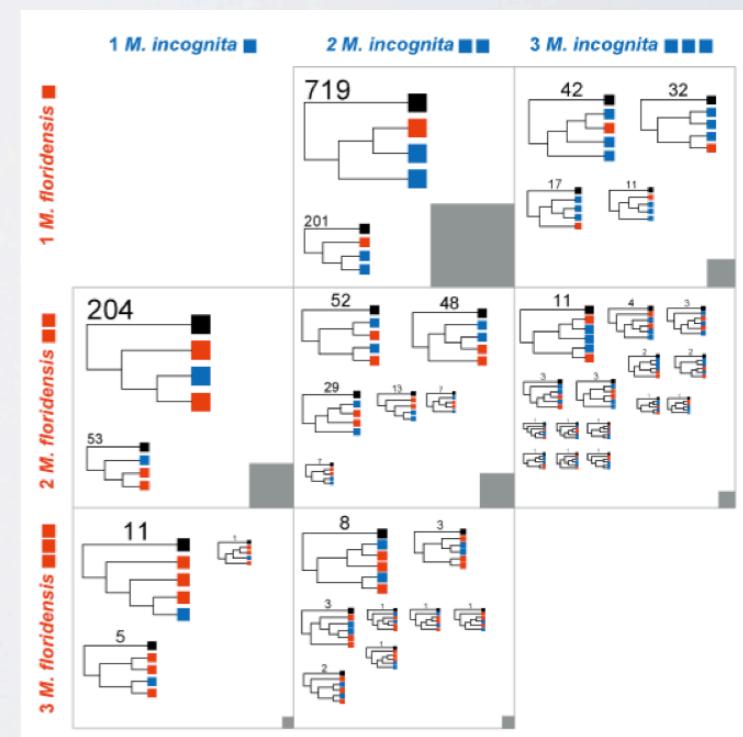
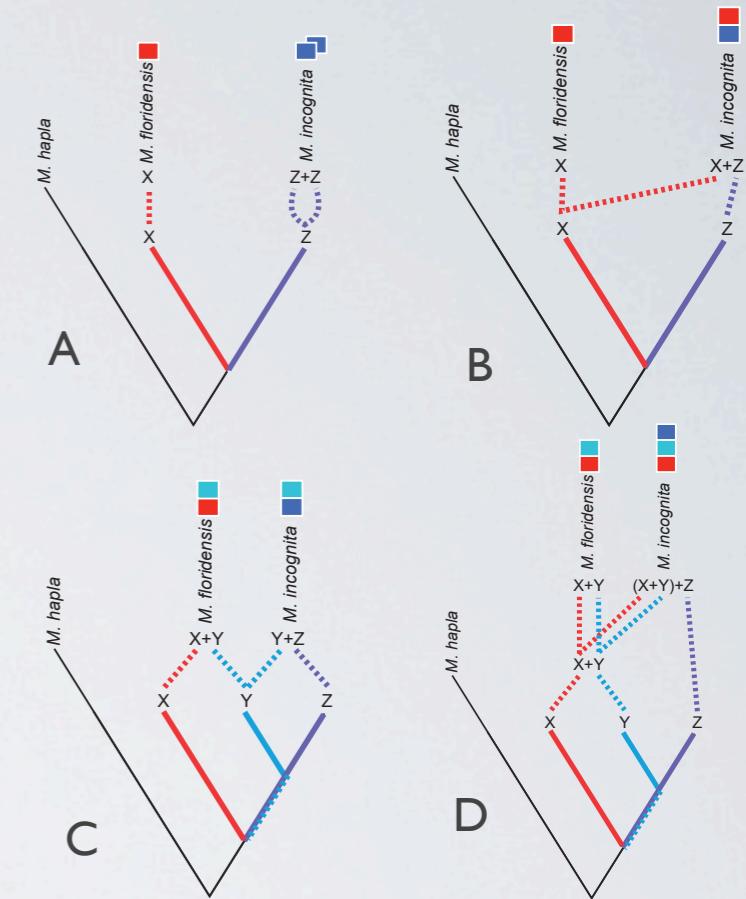
M. incognita and *M. floridensis* are independent hybrids sharing one parent



(D)
M. floridensis is a hybrid
 and *M. incognita* is a
 secondary hybrid
 between *M. floridensis* and
 a 3rd parent

Testing by Phylogenomics

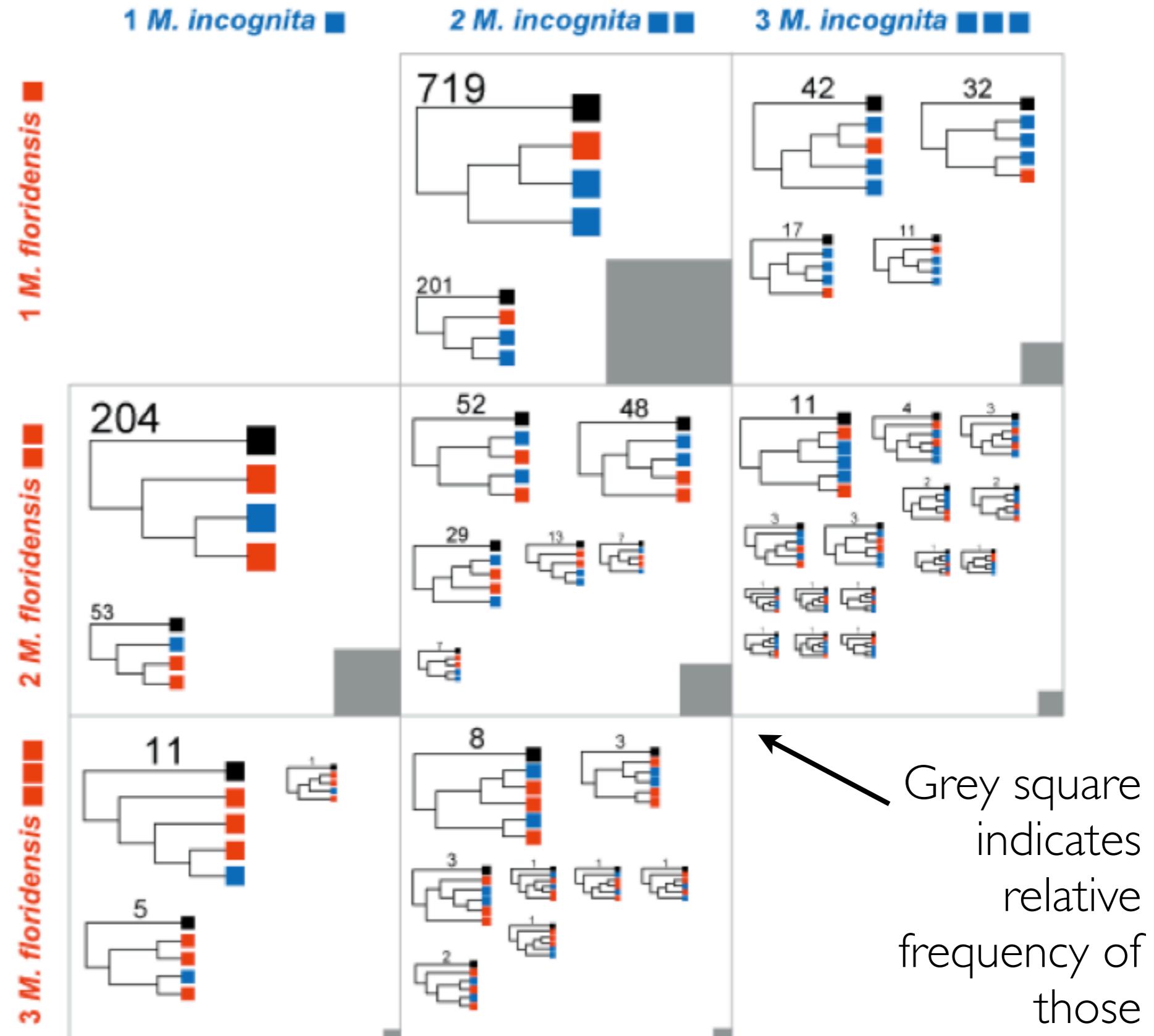
- Coding sequences from 3 genomes were placed into orthologous groups (InParanoid)
- 4018 ortholog clusters included all 3 species
- We retained those with a single copy in the outgroup *M. hapla*
- Phylogenies of relationships between Mi and Mf gene copies (RAxML)
- Trees were parsed and pooled to represent frequencies of different relationships



Each tree contains a single *M. hapla* sequence as outgroup (black square)

Grid squares represent different numbers of gene copies

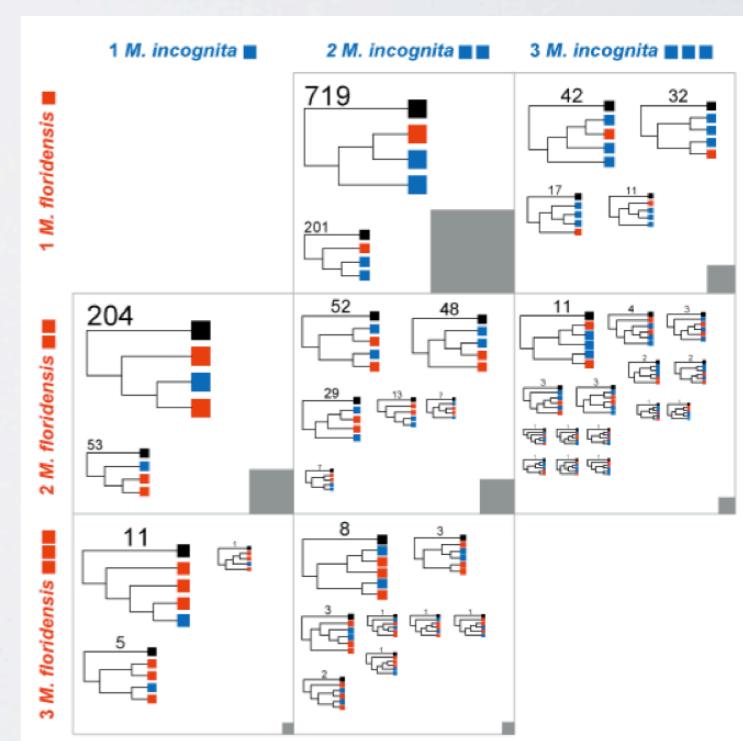
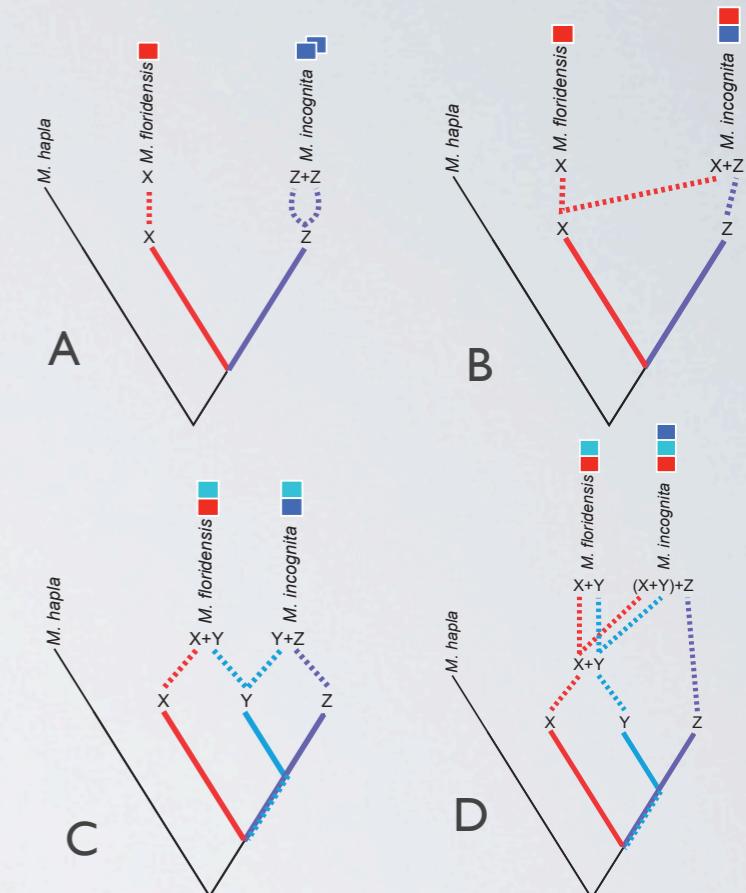
Trees are pooled within squares into different patterns of relationships



Grey square indicates relative frequency of those topologies

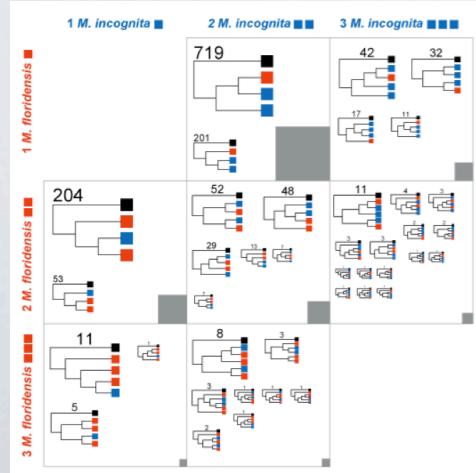
Testing by Phylogenomics

- We assess the fit of the tree topologies to our hypotheses
- Five out of seven cluster sets, and 95% of all trees, support hybrid origins for both *M. floridensis* and *M. incognita*
 - ie exclude hypotheses A and B
- Hypothesis C best explains 17 trees
- Hypothesis D best explains 1335 trees



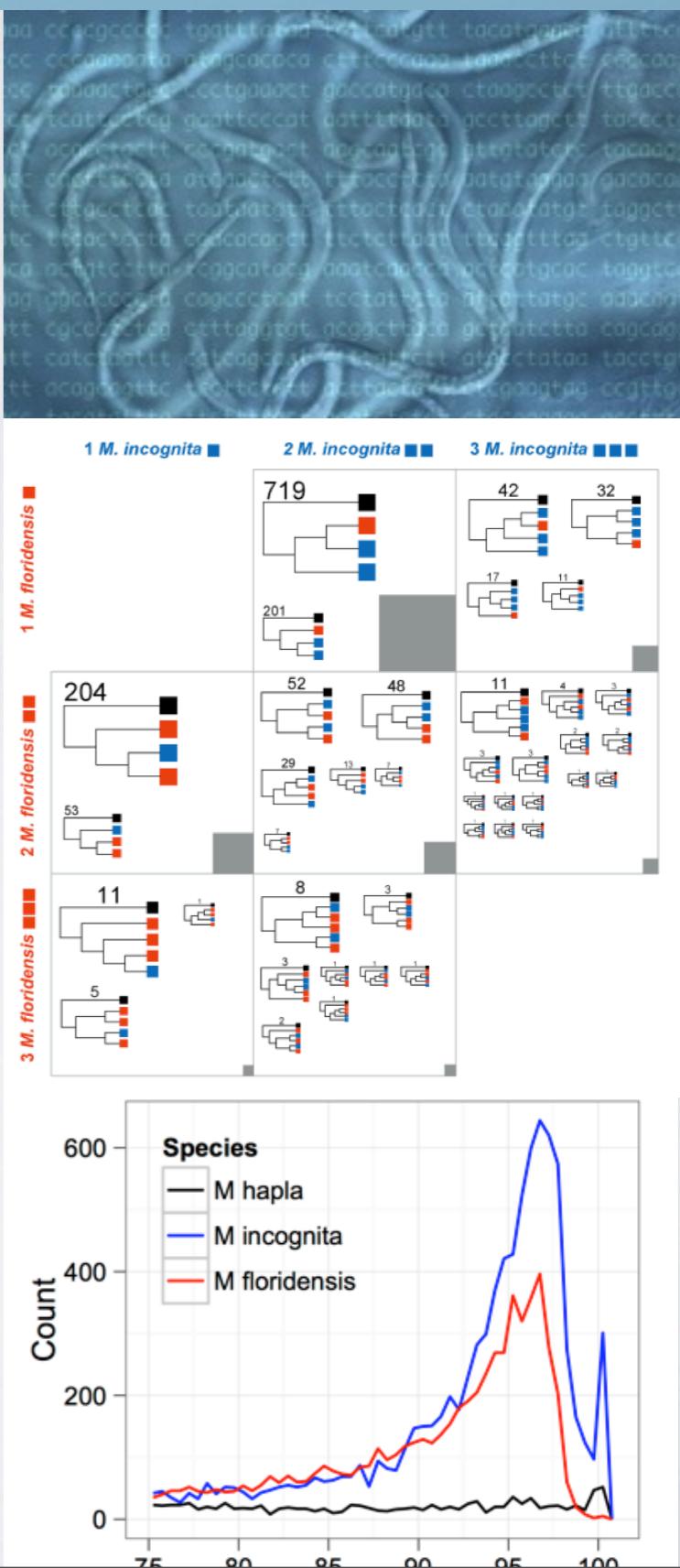
Testing by Phylogenomics

- The genome data supports both *M. incognita* and *M. floridensis* as interspecific hybrids
- M. floridensis* is a parental species of *M. incognita* with other parent unknown
- Complex hybridization may be a feature of this genus?



Comparative genomics questions

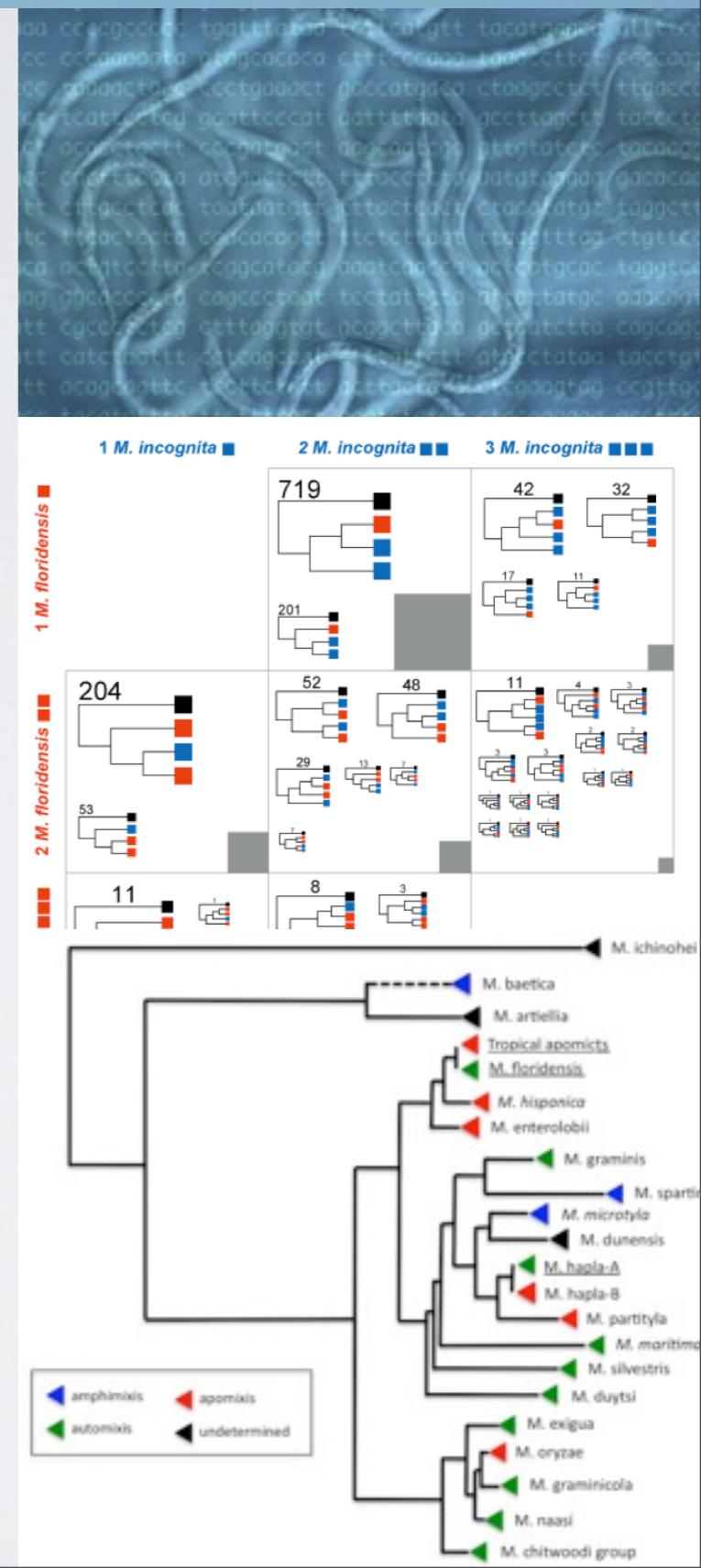
- Is there evidence of hybrid speciation?
 - Yes, complex hybrid origins are clear
 - Is *M. floridensis* a parental?
 - Yes, identified by phylogenomics and allelic sequence identity
 - How do offspring and parental genomes differ? What are the broader implications?
 - Ongoing work...



Ongoing Work

Current NERC grant on Meloidogyne breeding system and genome evolution

- 19 genomes in a phylogenetic design
- Testing effect of recombination & breeding system on genome change
 - hybrids, inbred, outbred, loss of meiosis
 - TEs, mutational patterns, gene families



Recombination and genomic rates and patterns of molecular evolution

COMPLEX HYBRID ORIGINS OF ROOT KNOT NEMATODES

Dave Lunt

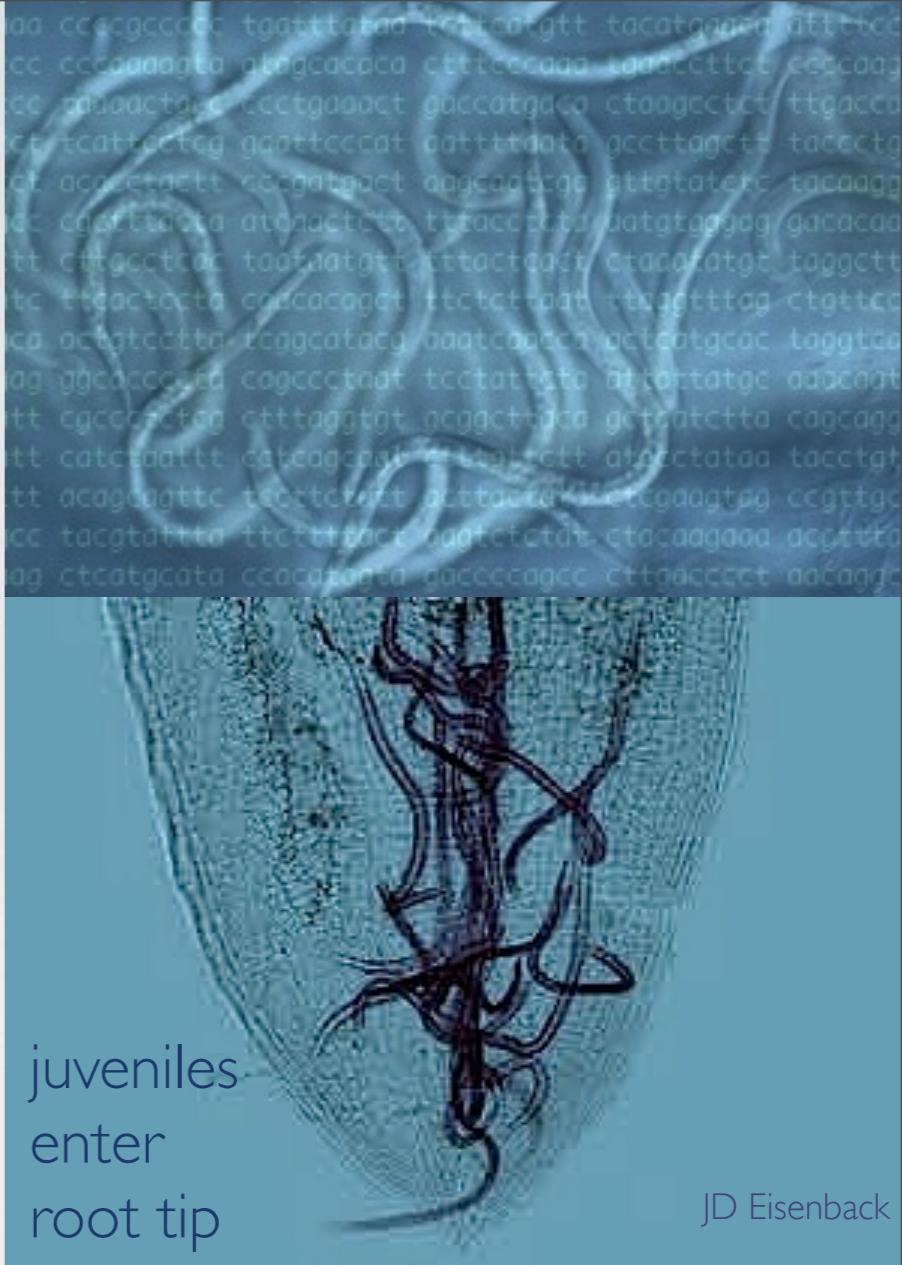
Evolutionary Biology Group, University of Hull

Sujai Kumar

Georgios Koutsovoulos

Mark Blaxter

Institute of Evolutionary Biology, University of Edinburgh



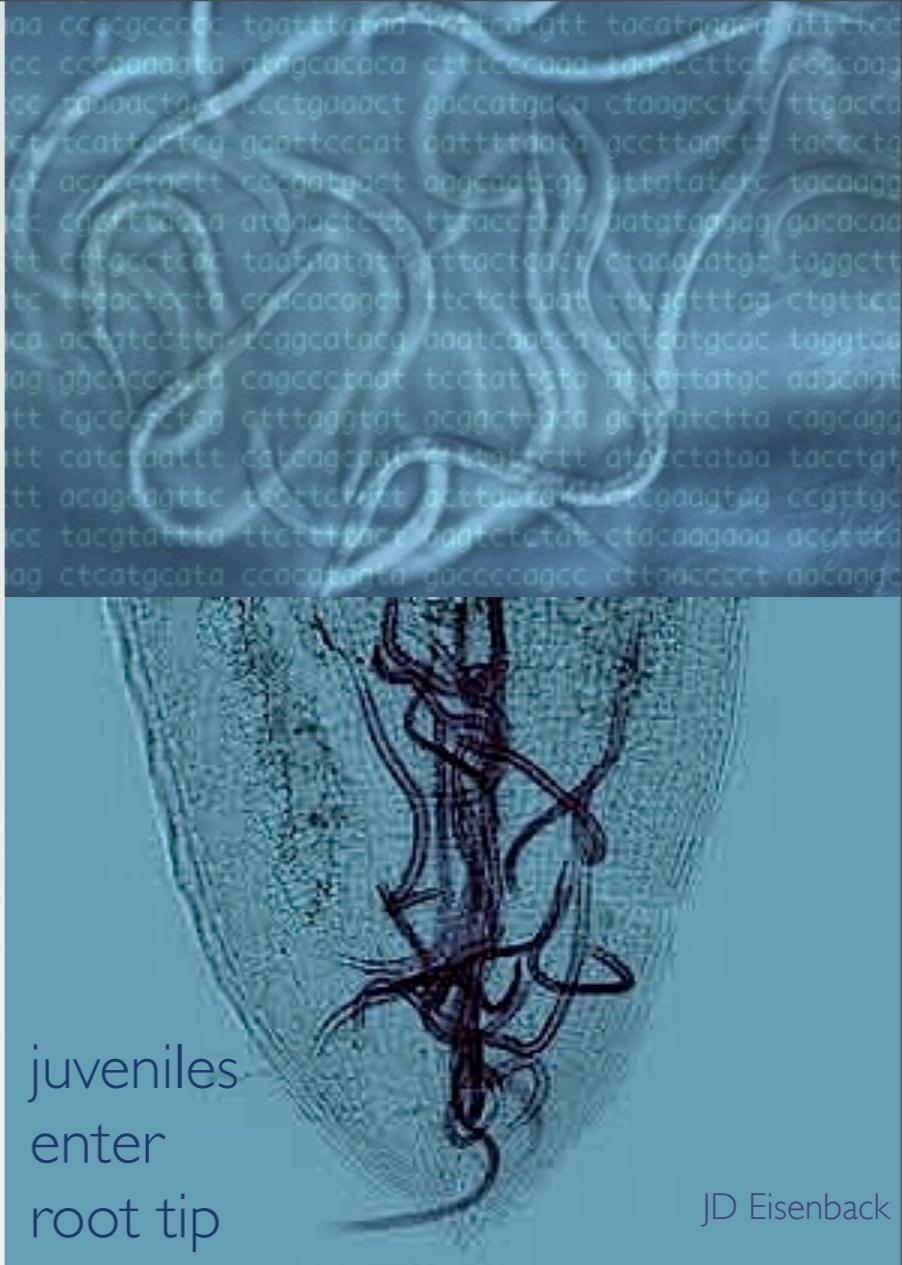
COMPLEX HYBRID ORIGINS OF ROOT KNOT NEMATODES

Dave Lunt
Evolutionary Biology Group, University of Hull

 @EvoHull  +EvoHull

 davelunt.net  dave.lunt@gmail.com

 @davelunt  +davelunt



Mark Blaxter

Institute of Evolutionary Biology, University of Edinburgh

 nematodes.org  mark.blaxter@ed.ac.uk



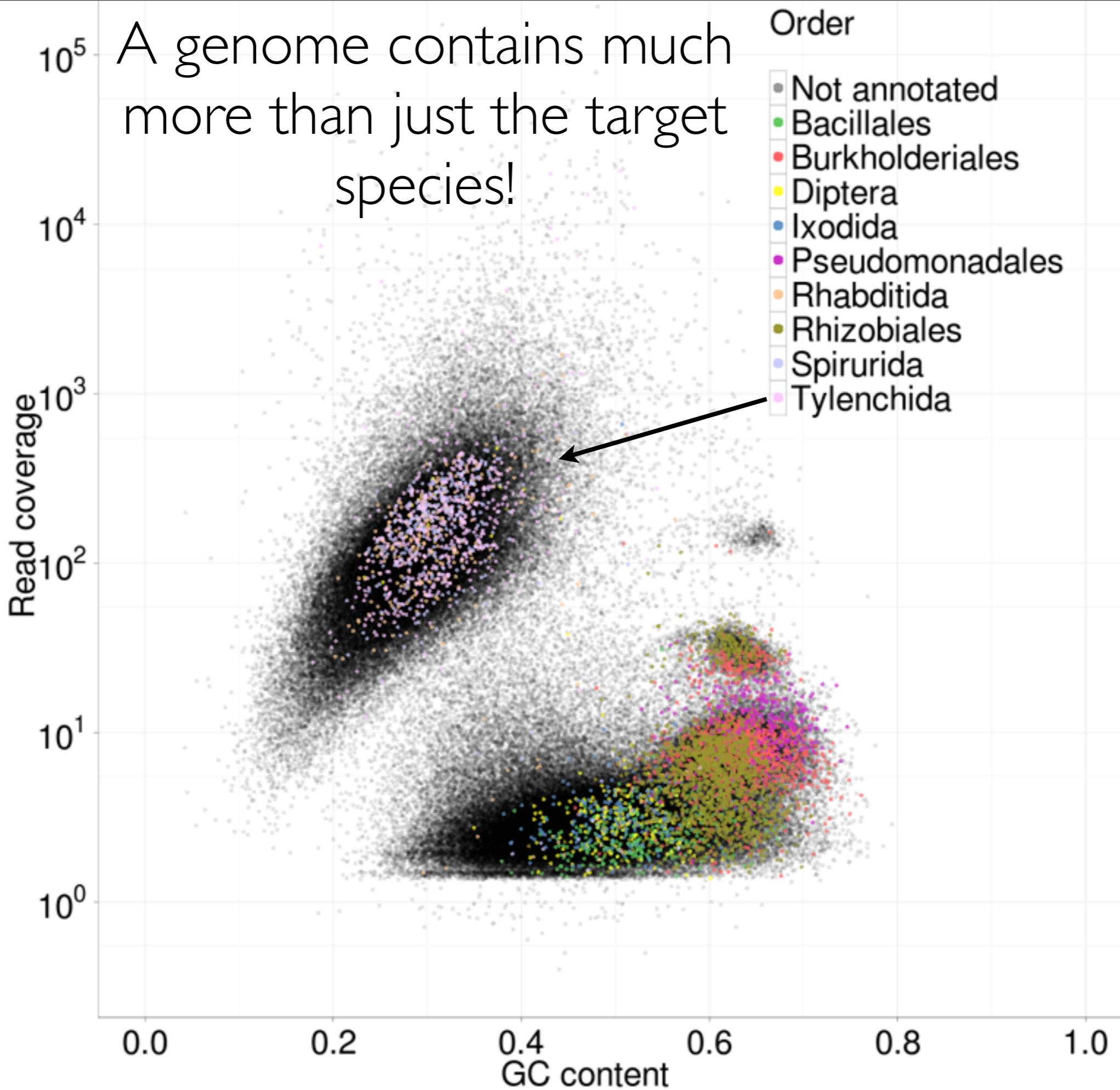
 **slideShare**
Present Yourself

<http://www.slideshare.net/davelunt/lunt-nottingham>

 **github**
SOCIAL CODING

<http://www.github.com/davelunt>

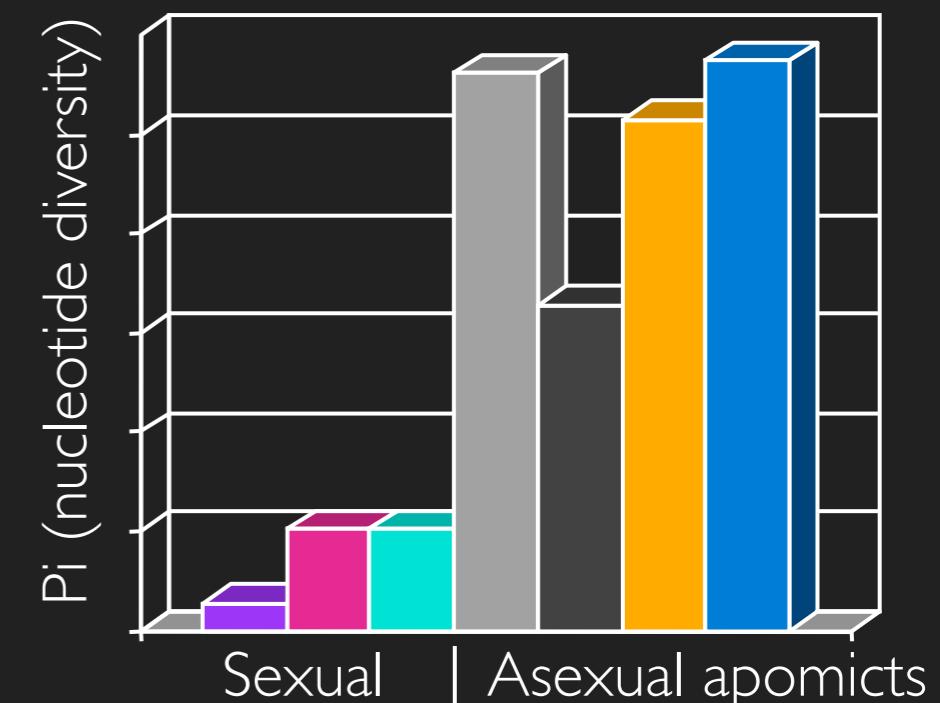
10^5 A genome contains much more than just the target species!



ASD IN SEXUAL AND ASEXUALS

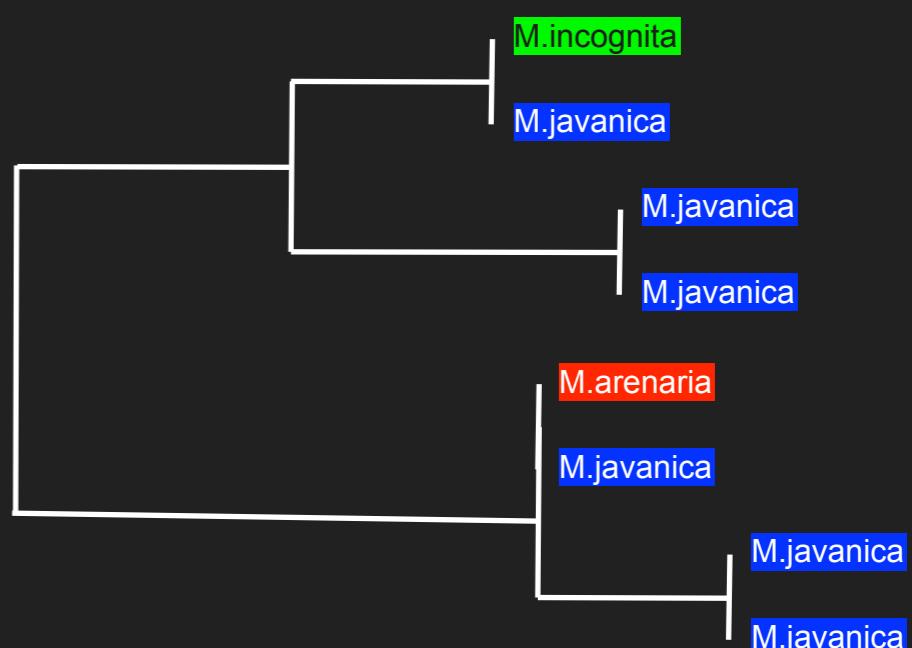
- Allelic Sequence Divergence levels are much greater in asexuals than sexuals
 - ASD can be very large within asexual individuals
 - Yet identical alleles found between different species

Nuclear protein-coding genes

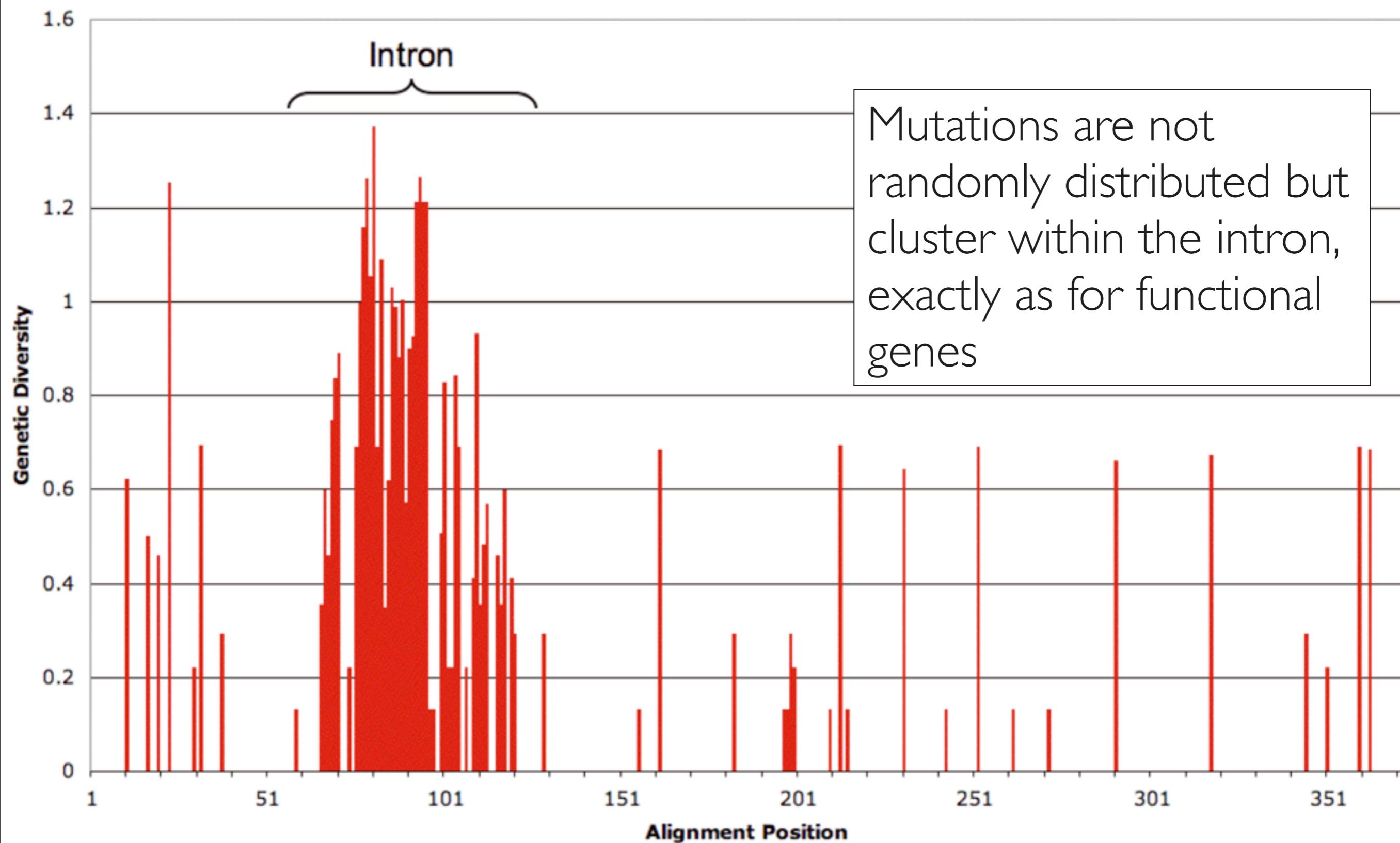


RNA polymerase II		
Species	Max intraspecific substitutions	Substitutions to closest relative
<i>M. incognita</i>	15	0 <i>M. javanica</i>
<i>M. javanica</i>	16	0 <i>M. incognita</i>

Dystrophin		
Species	Max intraspecific substitutions	Substitutions to closest relative
<i>M. javanica</i>	30	0 <i>M. arenaria</i>
<i>M. arenaria</i>	32	0 <i>M. javanica</i>



msp intron diversity in asexuals



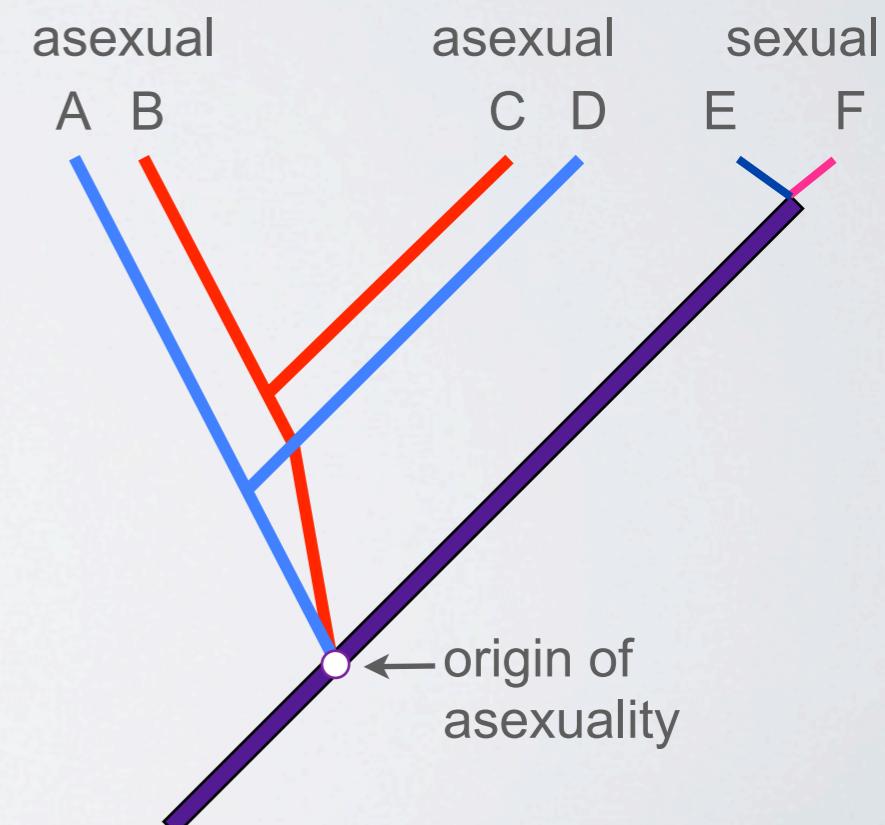
Selection on this gene cannot have been abandoned anciently

Extreme Allelic Sequence Divergence

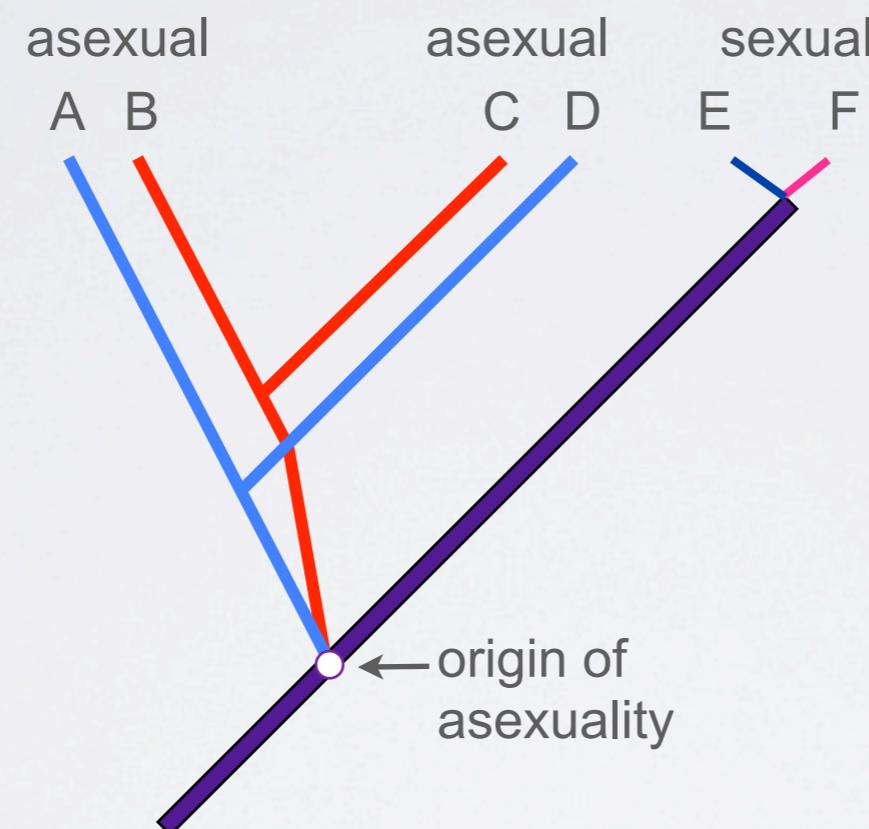
MJD White 'Animal Cytology and Evolution' 1st ed 1945, p283

"If we suppose an ameiotic form evolving for a very long period of time we might imagine its two chromosome sets becoming completely unlike, so that it could no longer be considered as a diploid either in a genetical or cytological sense."

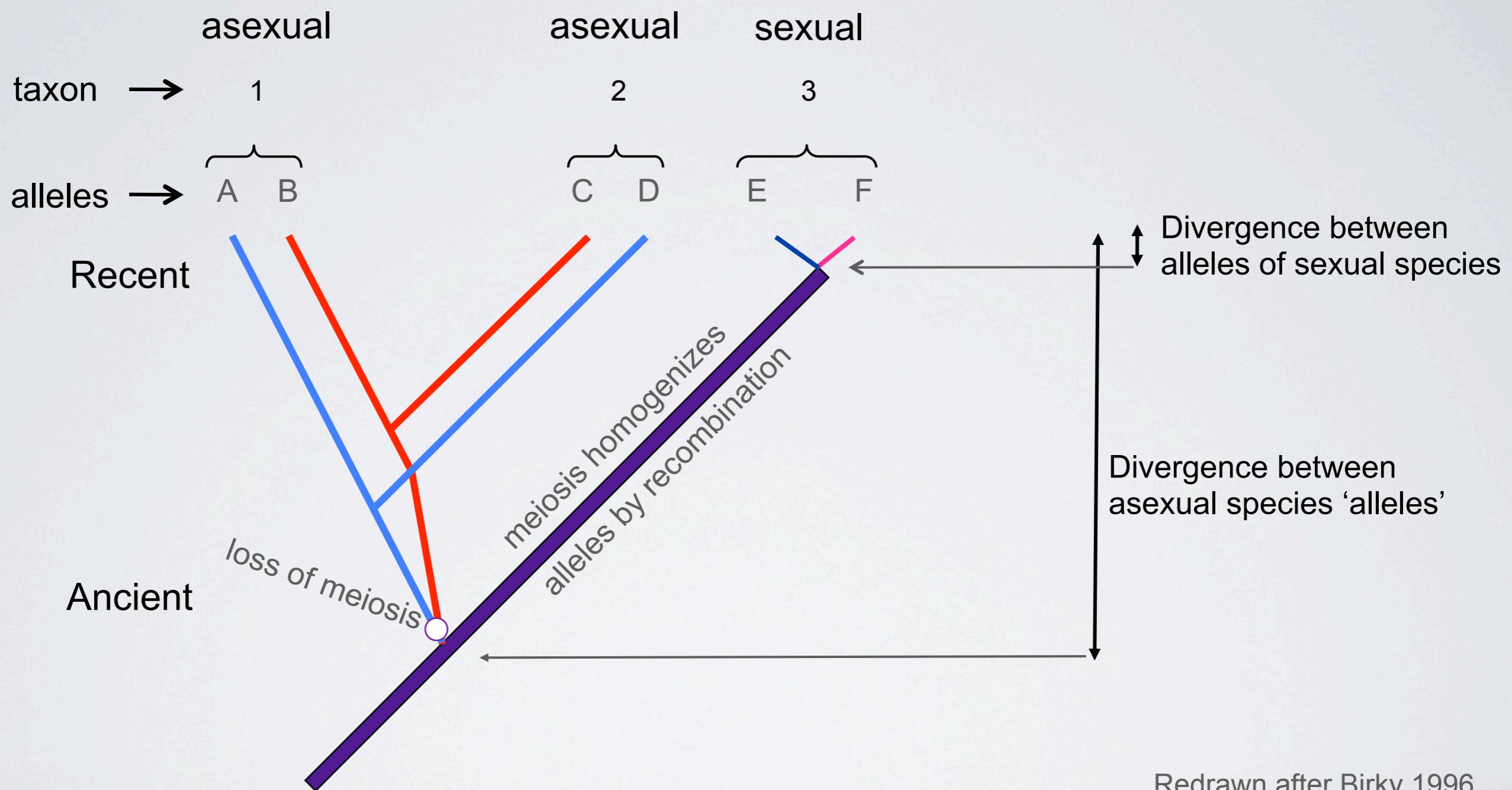
Sometimes called Meselson effect, similar to paralogous loci



Extreme Allelic Sequence Divergence

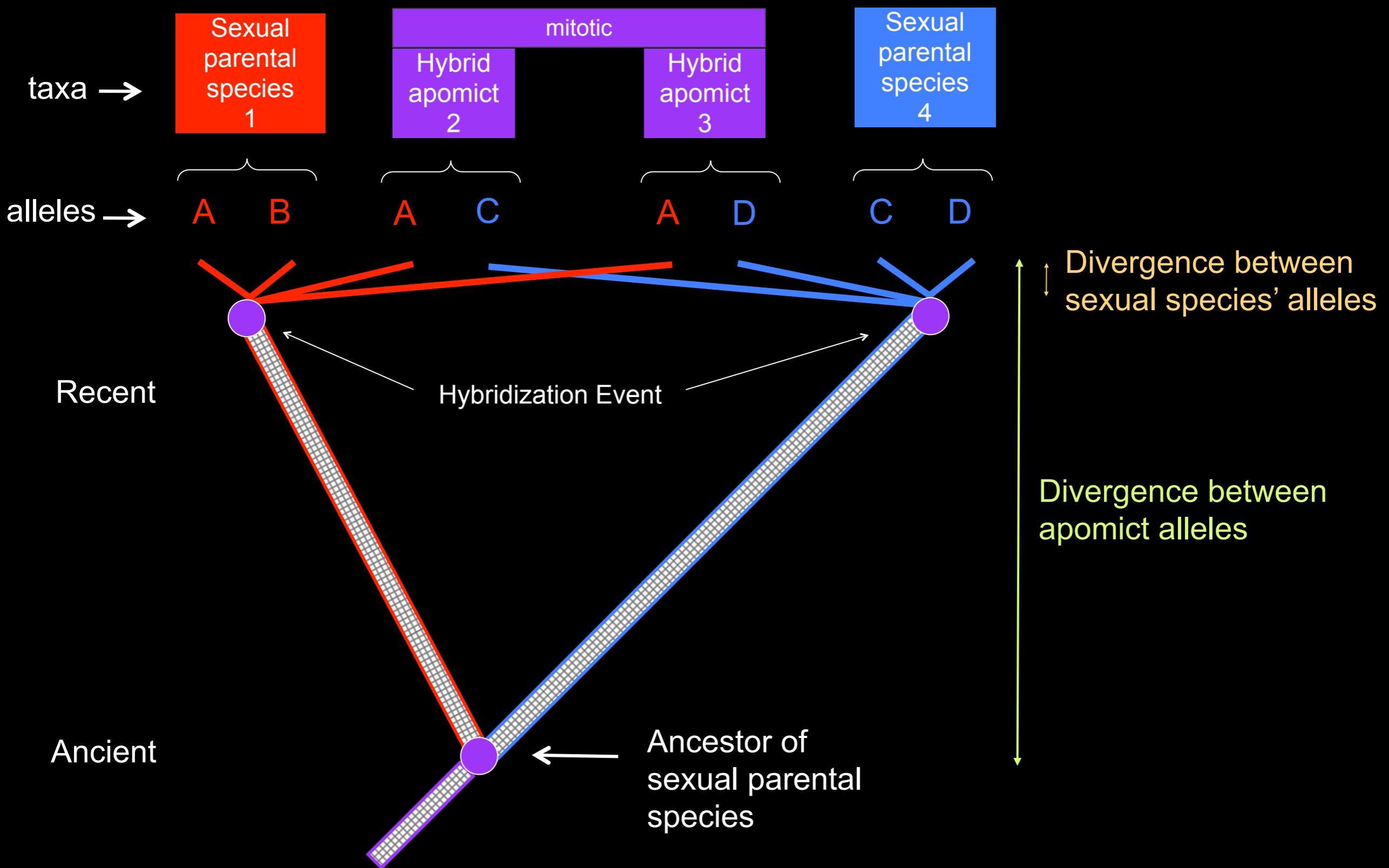


Extreme Allelic Sequence Divergence



Redrawn after Birk 1996

Recent interspecific hybridization



The *Meloidogyne floridensis* genome

- Stringent removal of bacterial sequences
- Clusters of bacterial orders
Bacillales, Burkholderiales,
Pseudomonadales and Rhizobiales
- Lower coverage and higher %GC clusters excluded
- Second round of megablast and hits to bacteria removed

