

# Novel transposons associated with *ToxA* and *ToxB* in the tan spot fungus

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Agriculture and  
Agri-Food Canada

Agriculture et  
Agroalimentaire Canada




# Tan spot of wheat

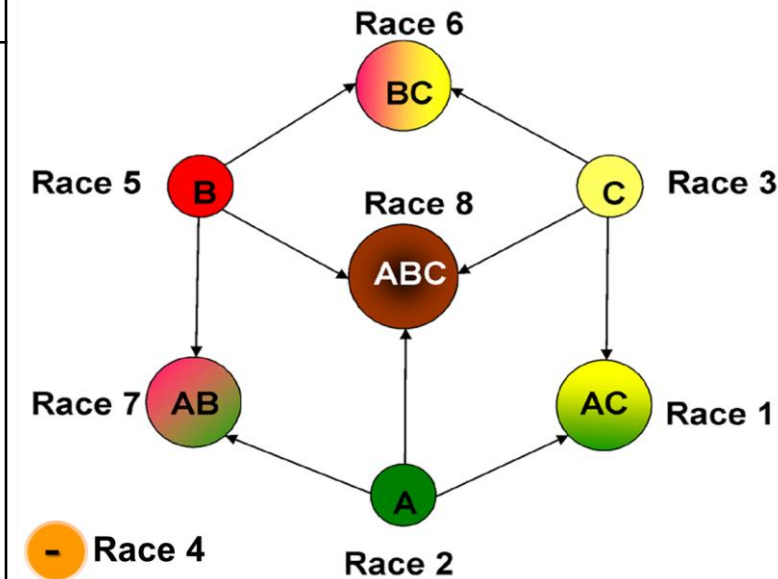
- *Pyrenophora tritici-repentis* (Ptr)
- Three necrotrophic effectors define current race system
- ~5% global losses<sup>1</sup>
- Recent emergence driven by *ToxA* HGT<sup>2,3</sup>

<sup>1</sup>Savary et al. 2019. Nat. Ecol. Evol. 3, 430-439

<sup>2</sup>Friesen et al. 2006. Nat. Genet. 38, 953-956

<sup>3</sup>McDonald et al. 2018. Mbio. 10, e01515-19

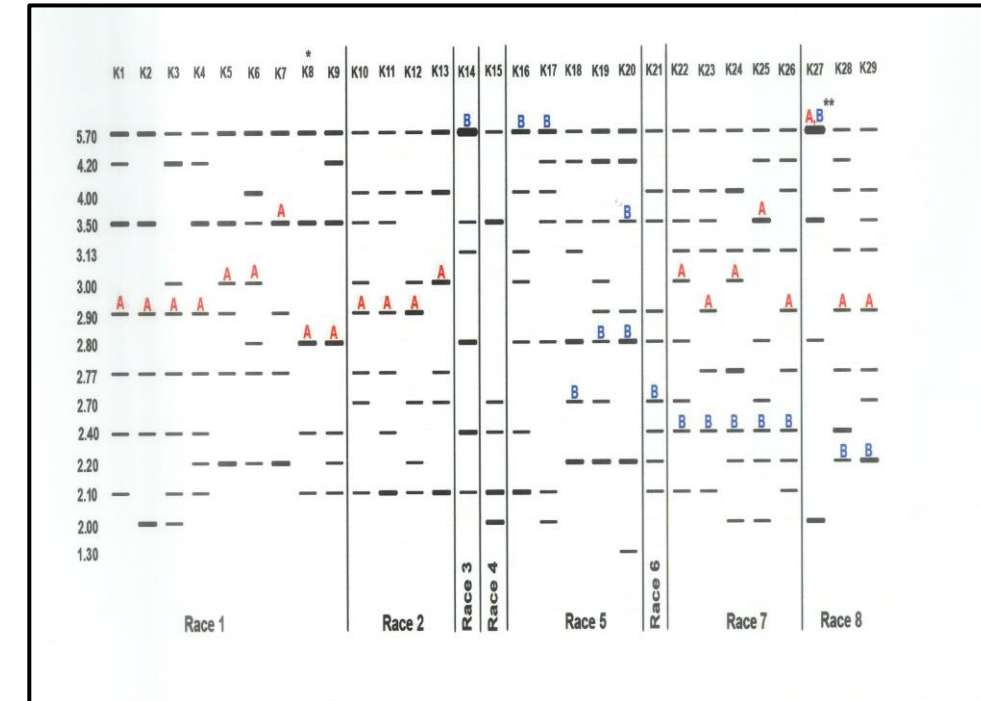
<i>ToxA</i>	<i>ToxB</i>	<i>ToxC</i>
Necrosis	Chlorosis B	Chlorosis C
		



Strelkov and Lamari, 2003. CJPP 25, 339-349

# Ptr genome

- Haploid ~35 to 40 Mb
- Primarily ToxA and ToxC producers sequenced
  - Recently many more released, and 40 of ours will be released soon
- Previous work with pulse field gel electrophoresis with all races showed:
  - plasticity in chromosome number and size
  - *ToxA* and *ToxB* never occurred on same chromosome
  - *ToxA* located on same essential chromosome with a couple of **exceptions**



Aboukhaddour et al. 2009. Mol. Plant Pathol. 10, 201-212

# Transposable elements

- Transposons are *mobile* genetic sequences (i.e they can alter position within a genome)
- Confer advantages (e.g drug resistance) or disease (e.g. porphyria)
- Some fungi have TE defense known as RIP (repeat induced point-mutations) (mutates C:G to T:A in repetitive regions)

## A unified classification system for eukaryotic transposable elements

Thomas Wicker, François Sabot, Aurélie Hua-Van, Jeffrey L. Bennetzen, Pierre Capy, Boulos Chalhoub, Andrew Flavell, Philippe Leroy, Michele Morgante, Olivier Panaud, Etienne Paux, Phillip SanMiguel and Alan H. Schulman

[Nature Reviews Genetics](#) **8**, 973–982 (2007)

## How TEs are classified

- RNA intermediate or DNA only
- ‘Copy & Paste’ or ‘Cut & Paste’
- Insertion mechanism
- Autonomous or non-autonomous
- Protein conservation + non-coding domains
- Presence and size of target site duplications
- DNA sequence conservation



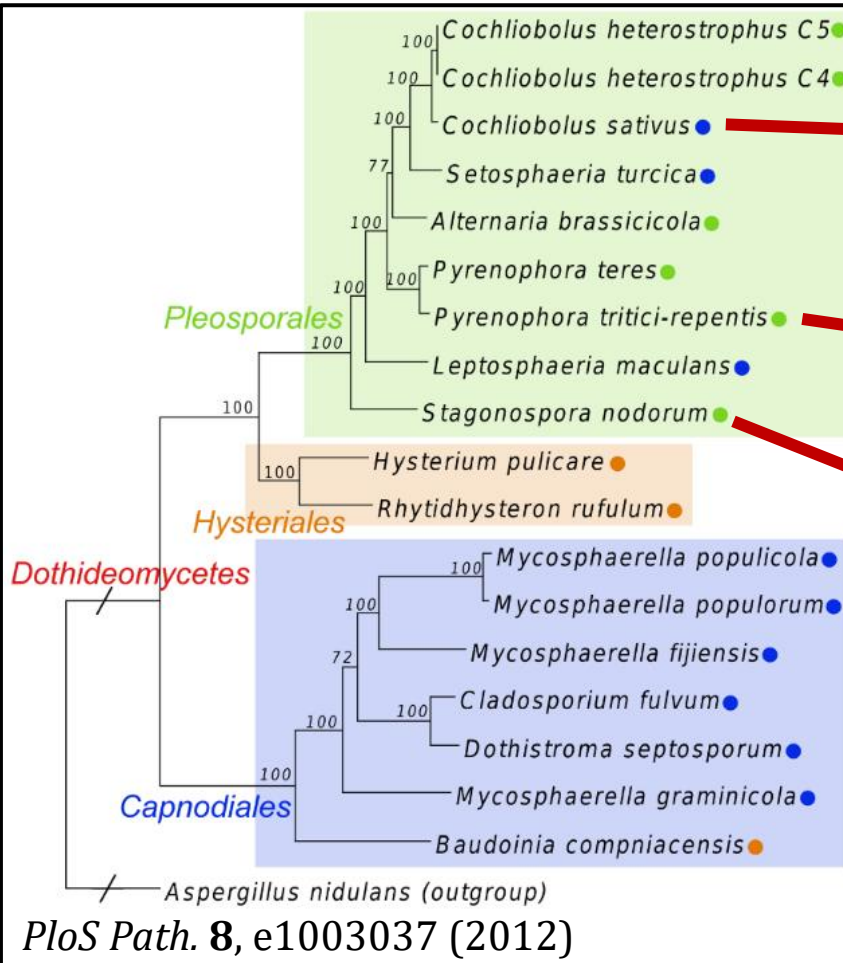
Barbara McClintock; Nobel Prize 1983



# TEs and pathogen virulence

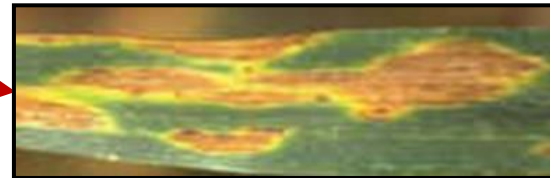
- Link between transposons and virulence is well established
  - 1980s prokaryote TE/HGTs explored extensively
  - 1990s TEs in eukaryotes and plant pathogens
  - 2000s '*two-speed*' model in fungal pathogens
  - 2010s long-reads available
  - 2020s beyond the two-speed model

# ToxA, HGT, and ToxhAT



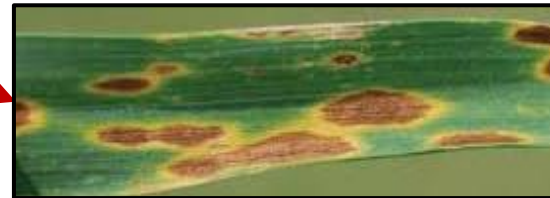
Spot blot

*Mol. Plant Pathol.* **19**, 432-439 (2018)



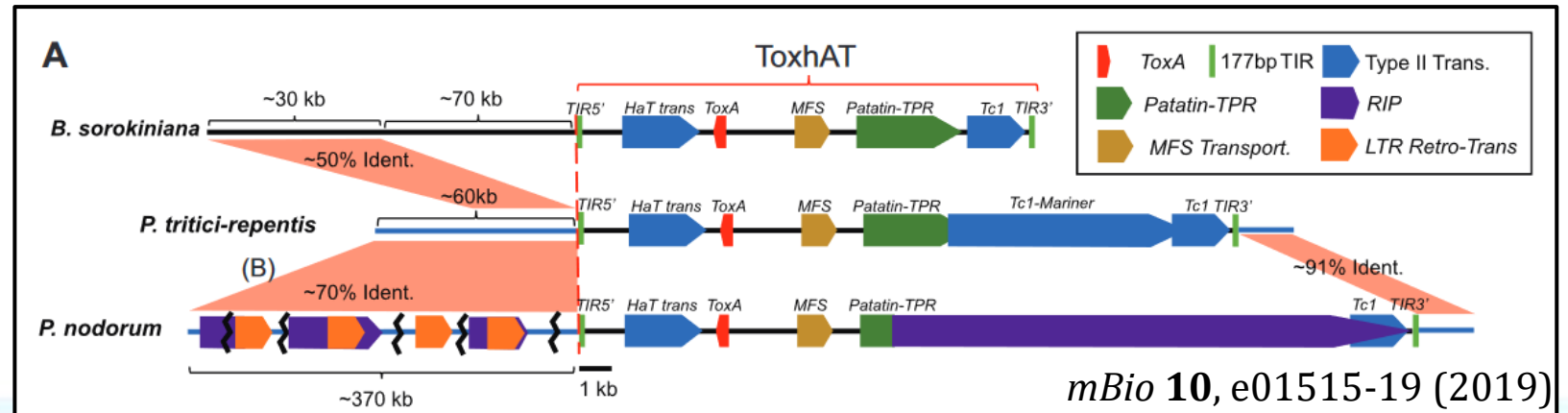
Tan spot

*Nat Genet.* **38**, 953-956 (2006)



Septoria blotch

*Nat Genet.* **38**, 953-956 (2006)



# *ToxB* in other species

See presentation by Mohamed Abdel-Fattah

“*ToxB* sequence diversity and haplotype network in *Pyrenophora tritici-repentis* and other Ascomycetes species”



# Objectives

- Produce high quality long-read assemblies of other races
- Confirm movement of *ToxA* within the Ptr genome
- Identify how *ToxA* moved within Ptr
- Determine if *ToxB* could be mobile
- Determine how *ToxB* replicates within Ptr
- Explore evolution of *ToxB* within Ptr and other species



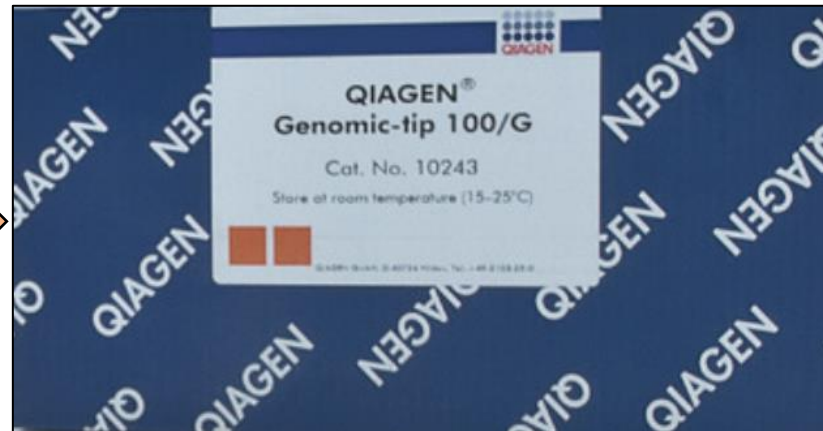
# Methods --- DNA & sequencing

Ptr grown in PDB flasks



race 8 and 3

DNA extraction



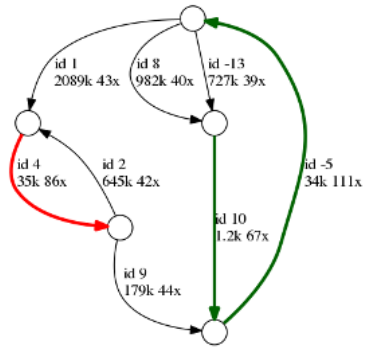
Seq with PacBio RS II



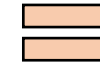
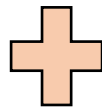
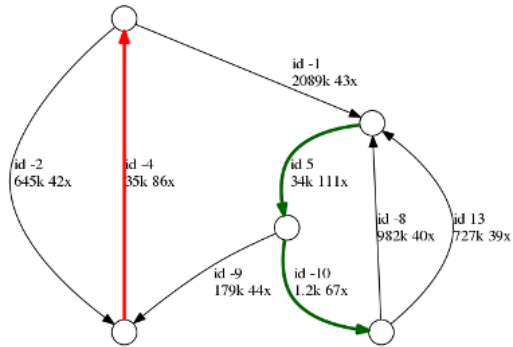
@ Genome Quebec

# Methods --- *de novo* assembly

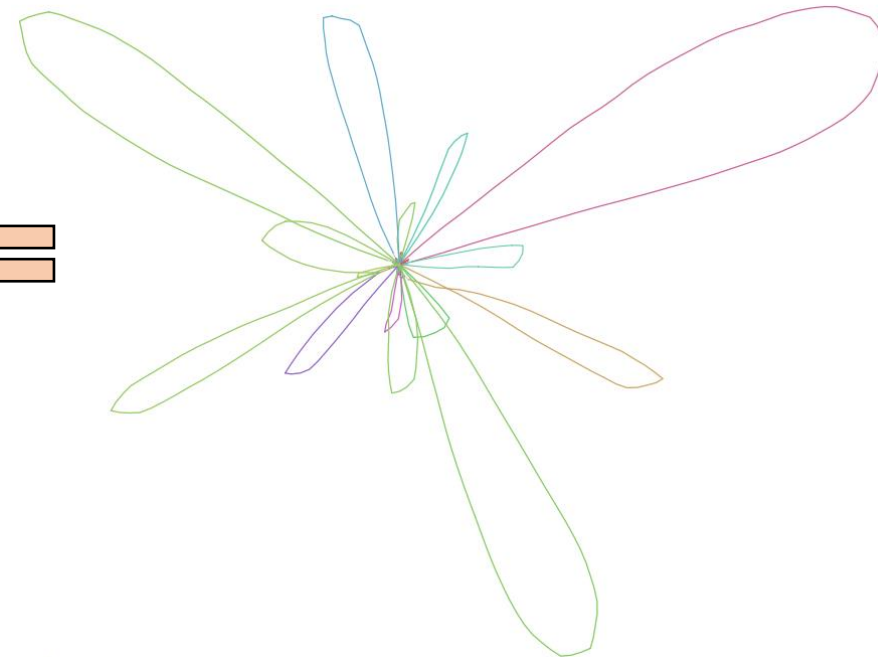
Flye assembler (repeat graphs)



Pilon (error correction)




Long-read assembly



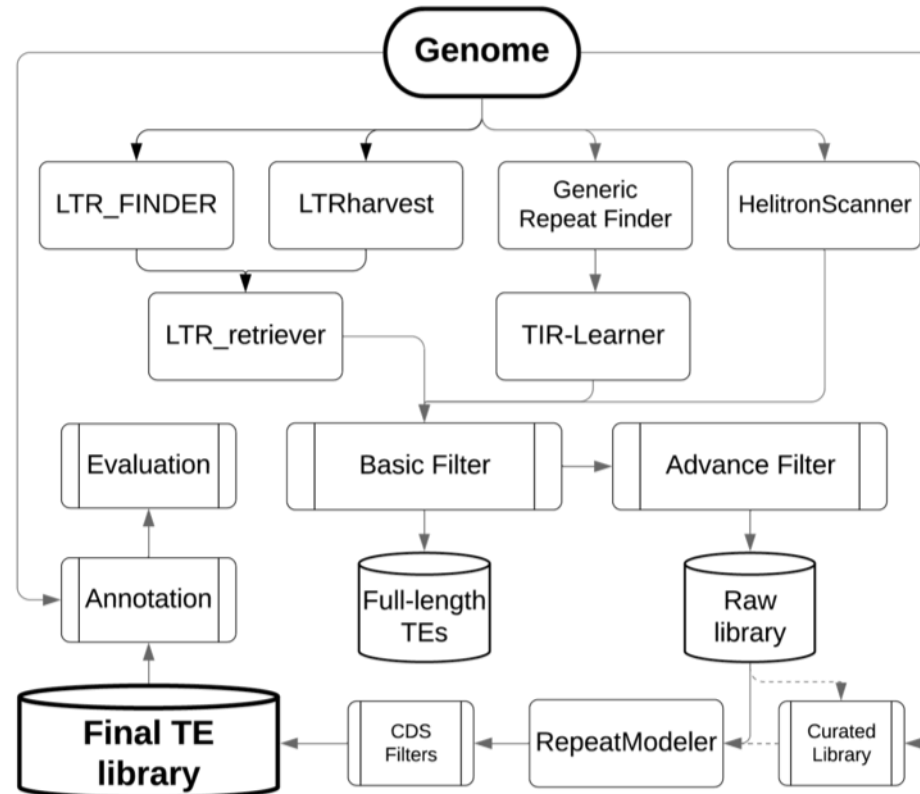
# Methods --- genome annotation

Gene annotations FunGAP: fungal Genome Annotation Pipeline

Protein annotations **Pfam** +  + **Phyre<sup>2</sup>**

Transposon annotations  
**oushujun/EDTA**

Extensive de-novo TE Annotator



# Methods --- alignments

Locate *ToxA* and *ToxB*



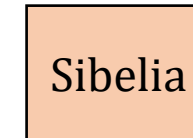
Extract *Tox* containing  
chromosomes



Linear alignments



Circular alignments





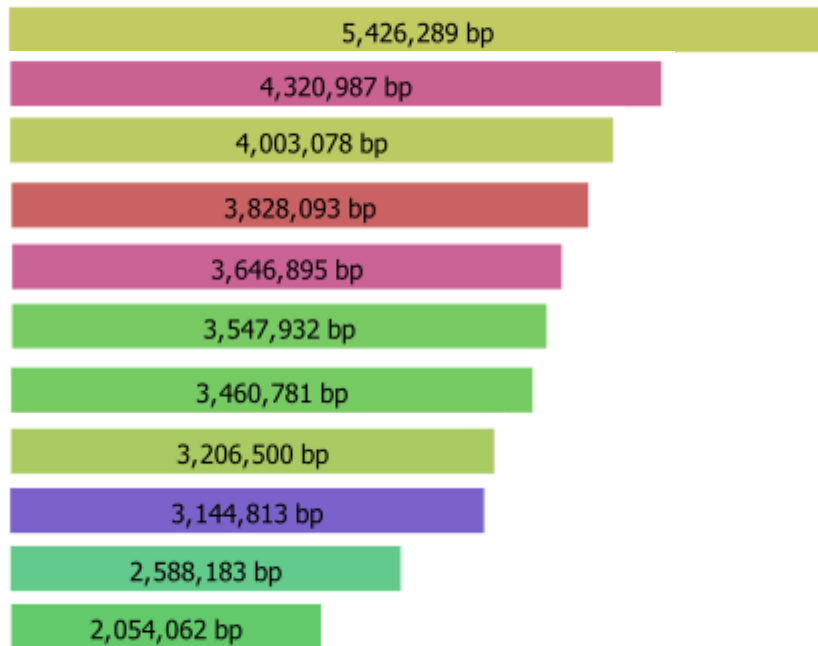
# Long-read assemblies

## I-73-1

--- R8 Syria

--- 39.9 Mb

--- 12,744 genes; BUSCO 99.6%



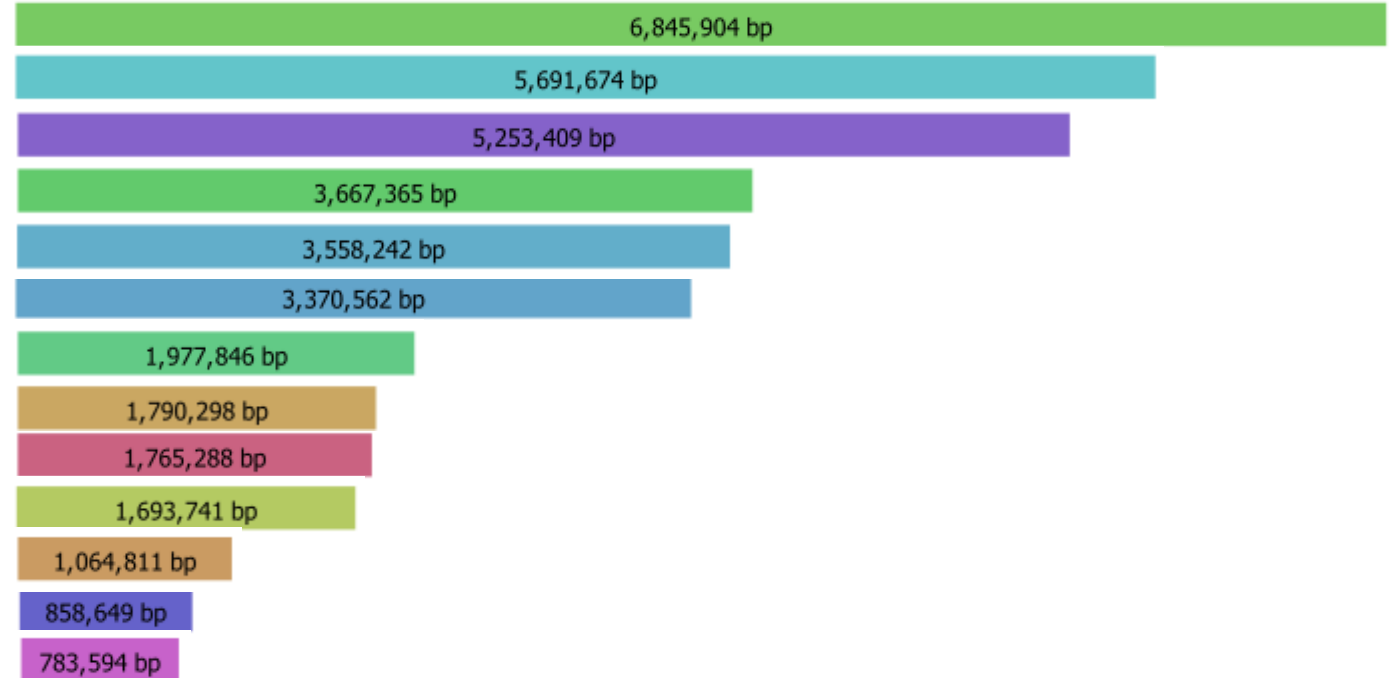
+28 smaller contigs

## D308

--- R3 Canada

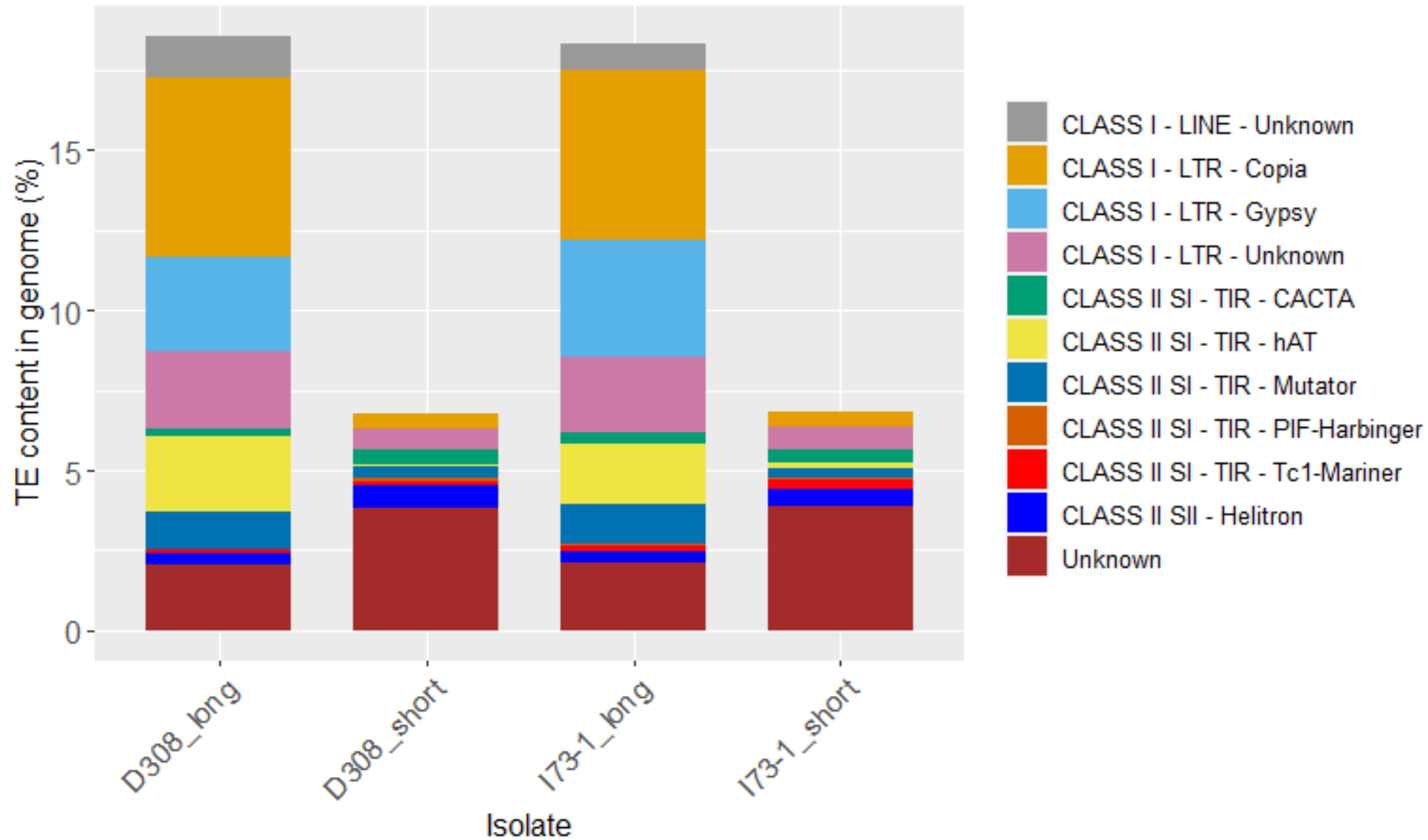
--- 39.6 Mb

--- 12,501 genes; BUSCO 99.6%

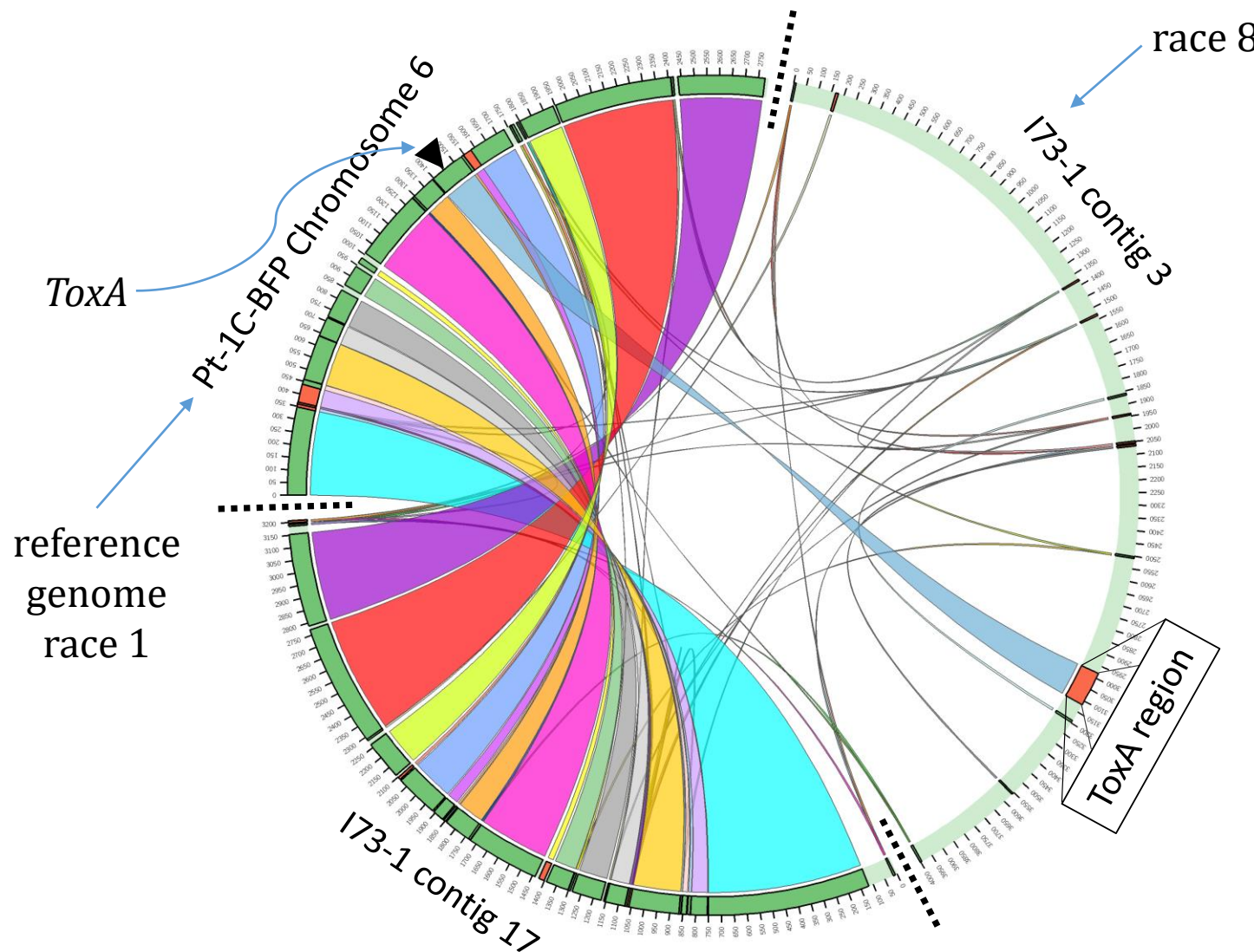


+57 smaller contigs

# Transposable element content



# Intraspecific *ToxA* translocation

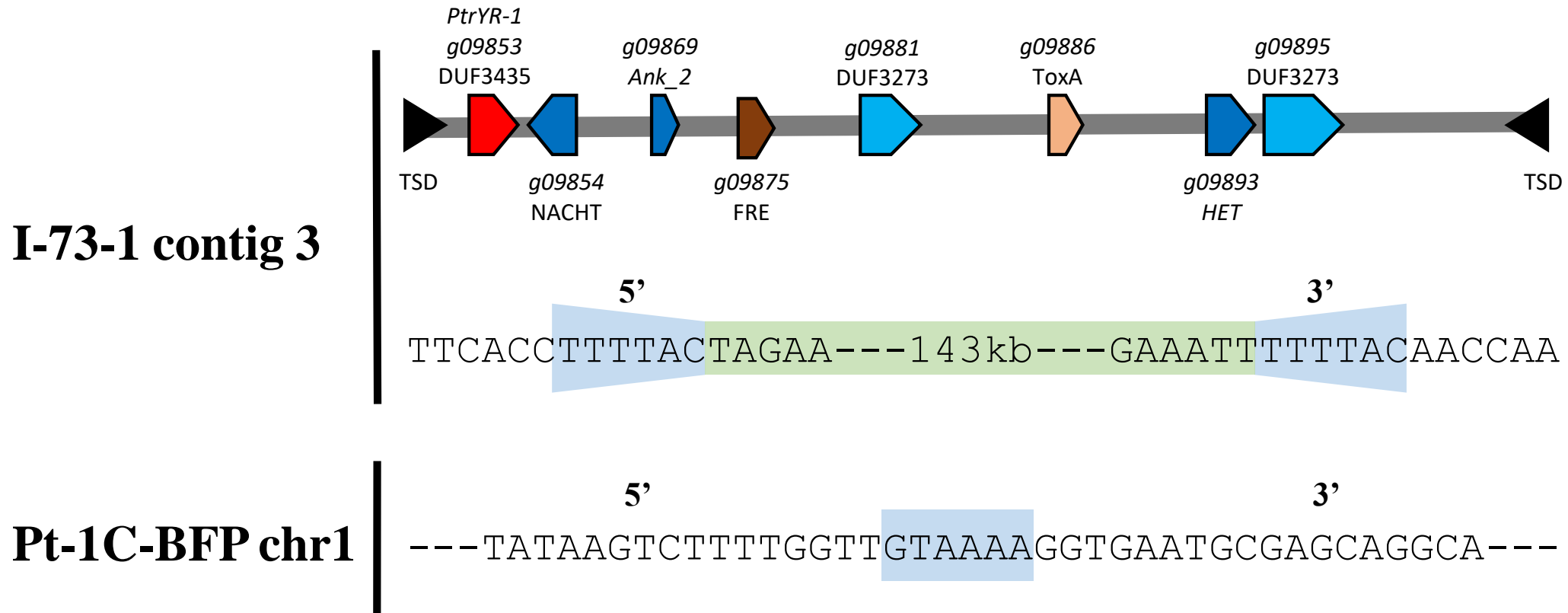


- Confirms work with PFGE<sup>1</sup>
- 143 kb element
- ToxhAT fully nested
- Nested transposons associated with rapid evolution<sup>2</sup>

<sup>1</sup>*Mol. Plant Pathol.* **10**, 201-212 (2009)

<sup>2</sup>*Mol. Sciences.* **20**, 3597 (2019)

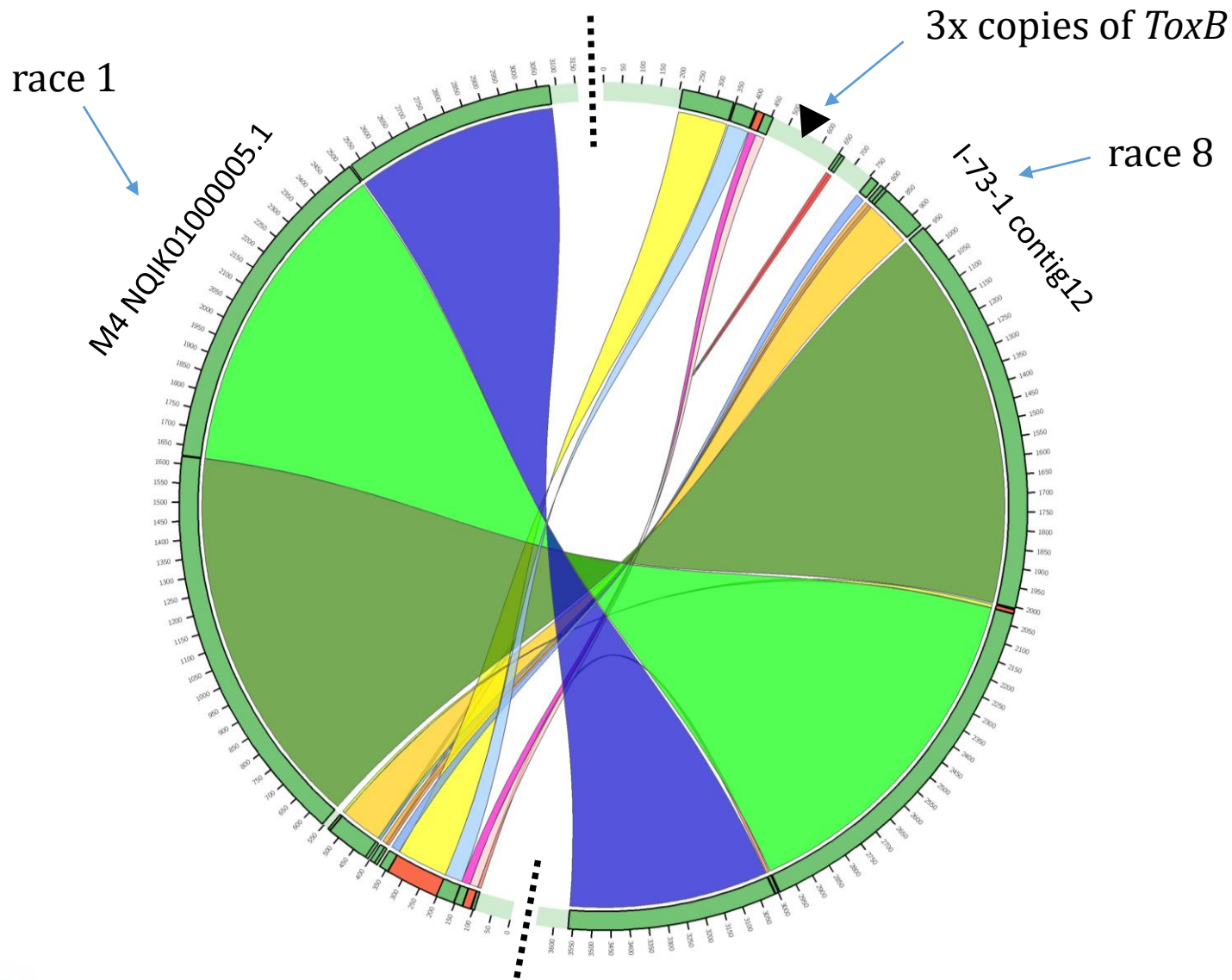
# *ToxA* Starship 'Horizon' schematic



Gluck-Thaler et al., 2021. Giant *Starship* elements mobilize accessory genes in fungal genomes. *bioRxiv* preprint doi:10.1101/2021.12.13.472469

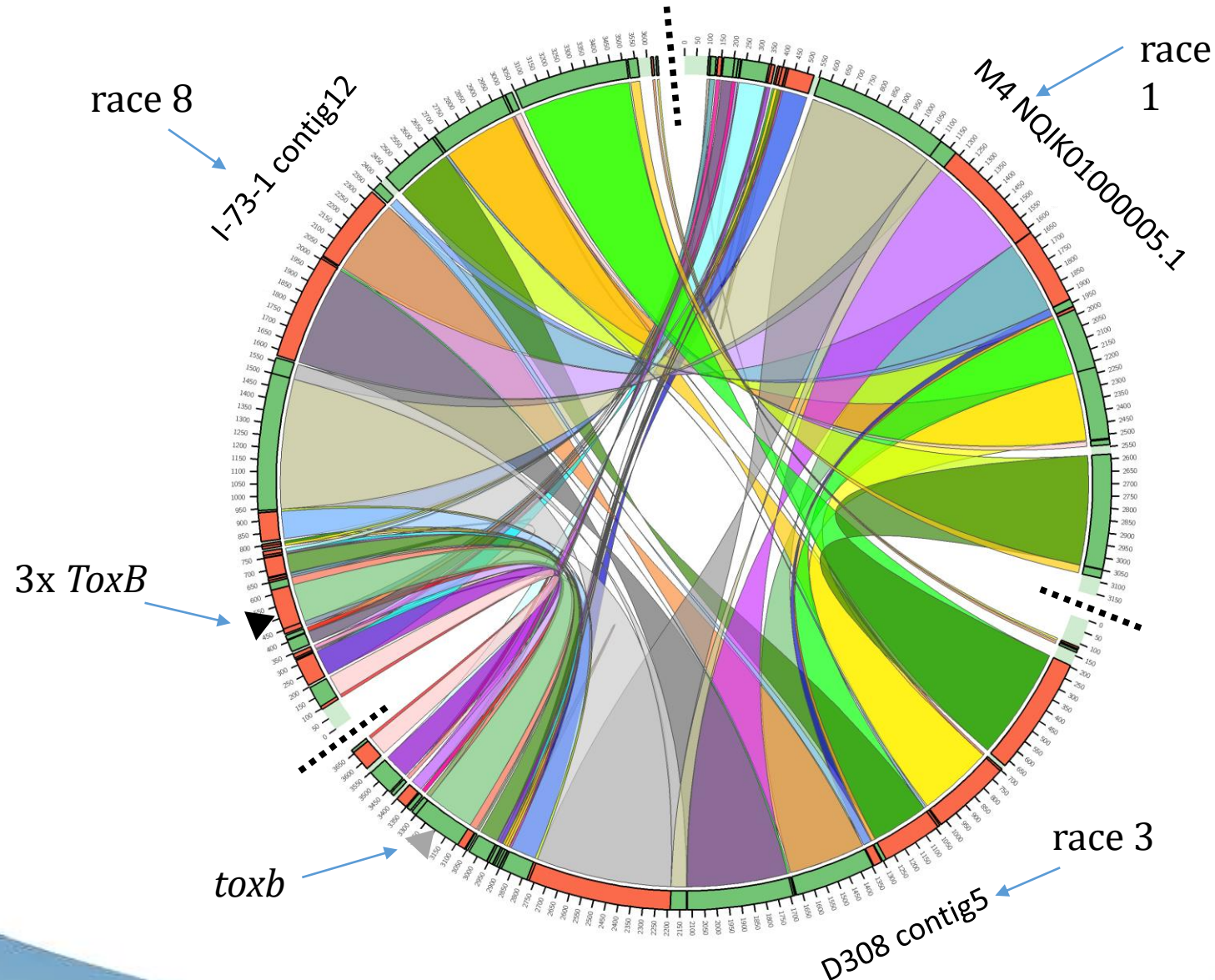


# *ToxB* on a putative transposon



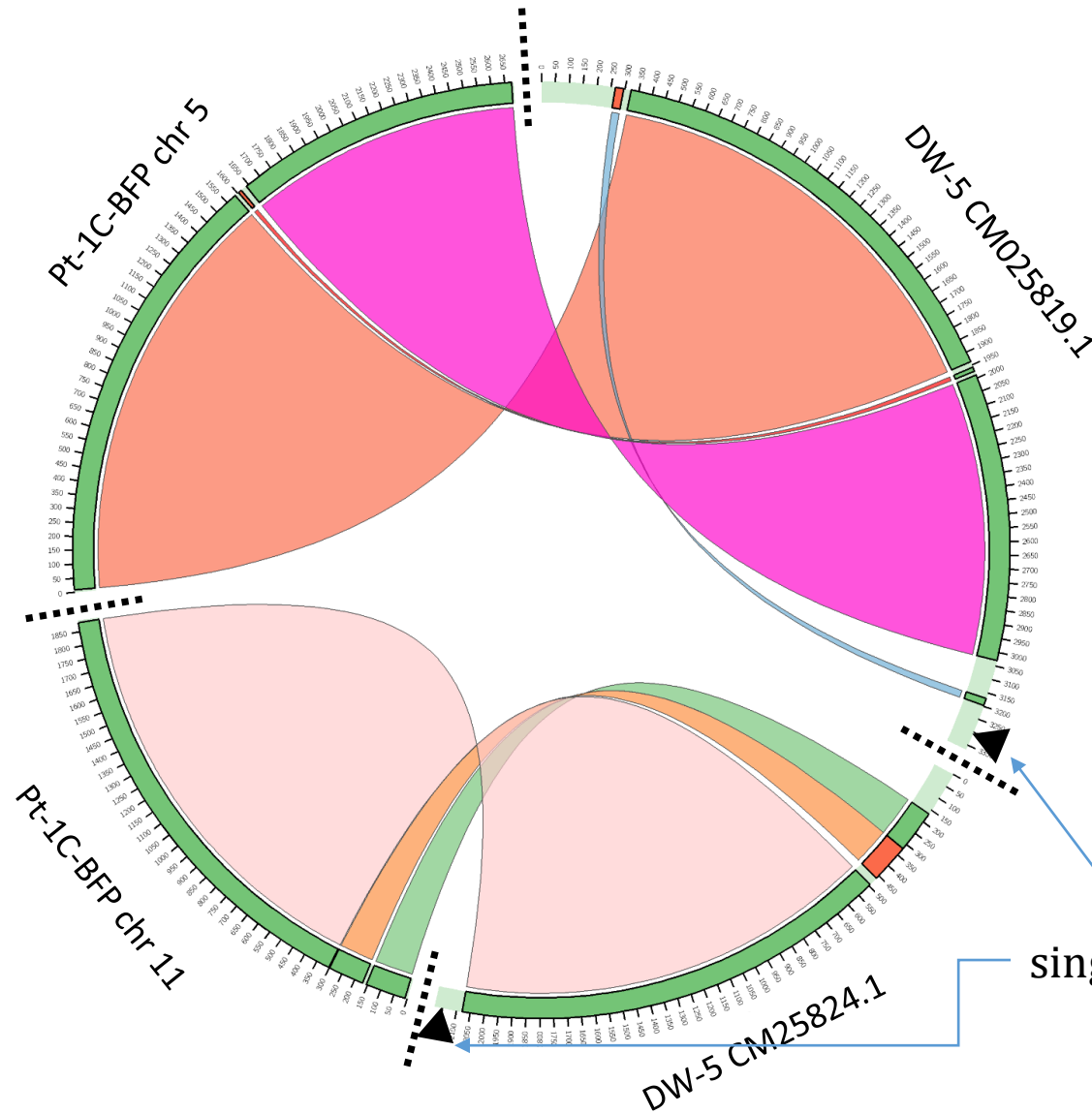
- 294 Kb element
- Small 12 Kb region contains 3x copies of *ToxB*
- Aligns nowhere in genome of race 1 isolates
- Edges appear to be terminal inverted repeats
- Target site visible in M4 race 1

# *ToxB* on a putative transposon



- Same element present in race 3 with *tox b*
- *tox b* in same general area but as single copy

# ToxB results



- Different elements containing single copies of *ToxB* in race 5
- Presence at the edges of contigs may indicate misassemblies

single copies of *ToxB*



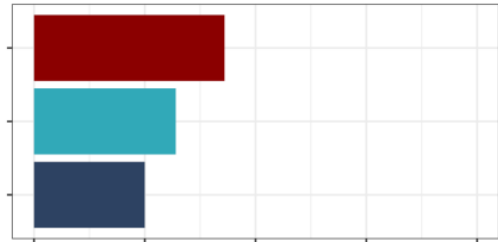
# Conclusions and future work

- First long-read assemblies of race 3 and race 8
- *ToxA* is nested within multiple transposons: ToxhAT and novel Starship 'Horizon'
- *ToxB* may also be present on massive transposon(s) and may also be or was mobile
- How is *ToxB* replicating? Good chance there is transposon involved!
- Has *ToxB* been involved with a horizontal gene transfer event?
- Recently received long-read data for 17 more isolates!
- Plans for long-read sequencing of other *ToxB* containing species

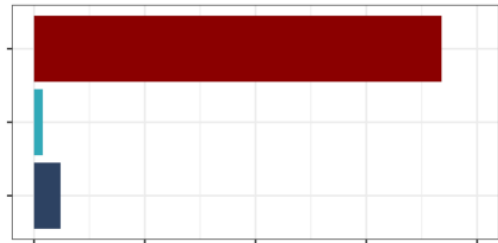


# Ptr pangenome figures

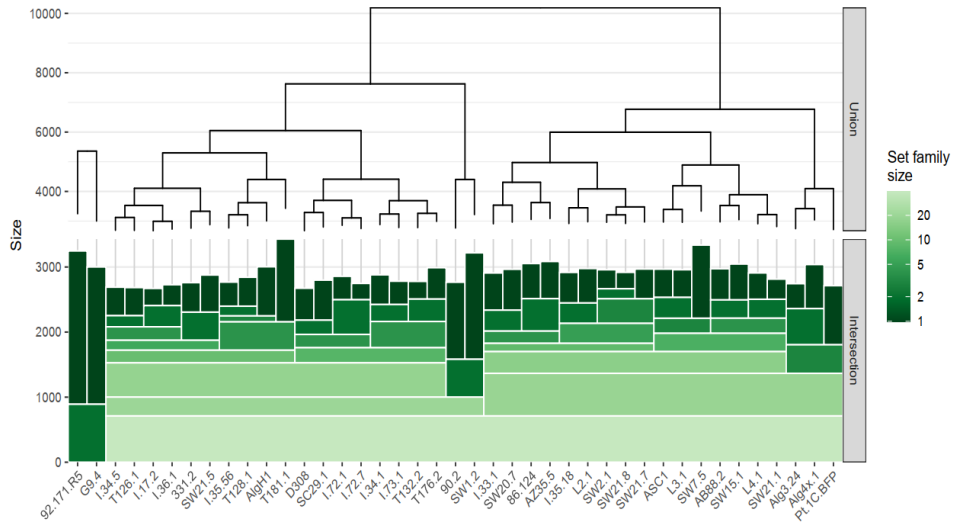
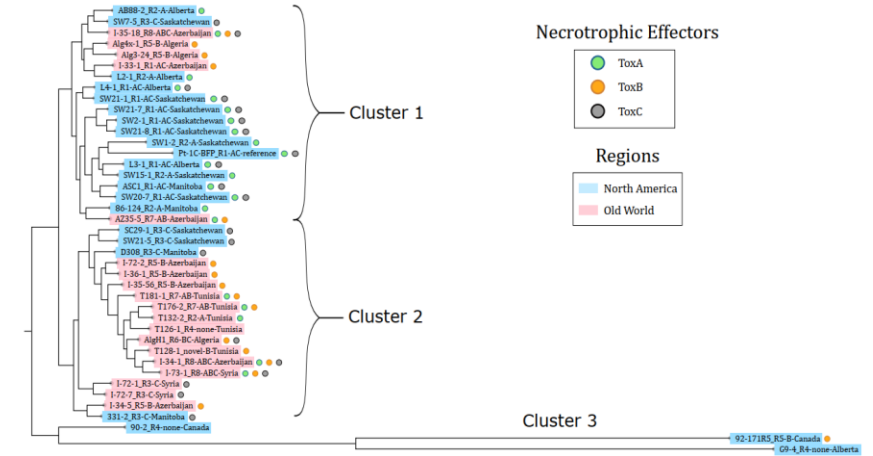
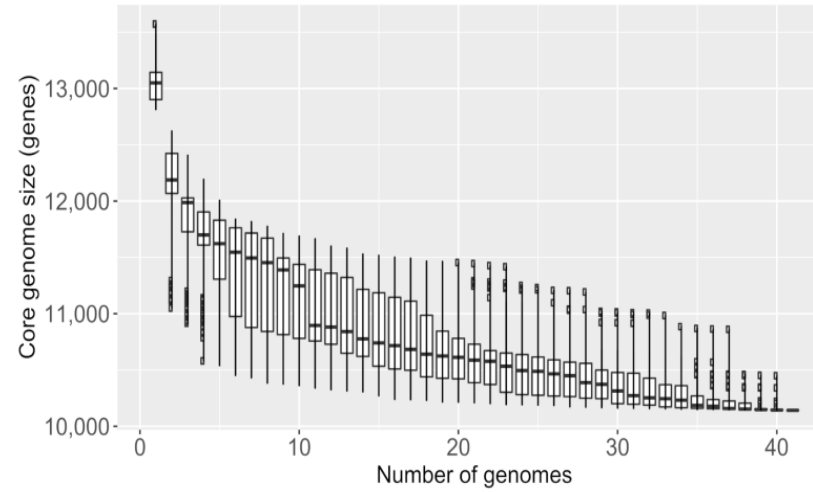
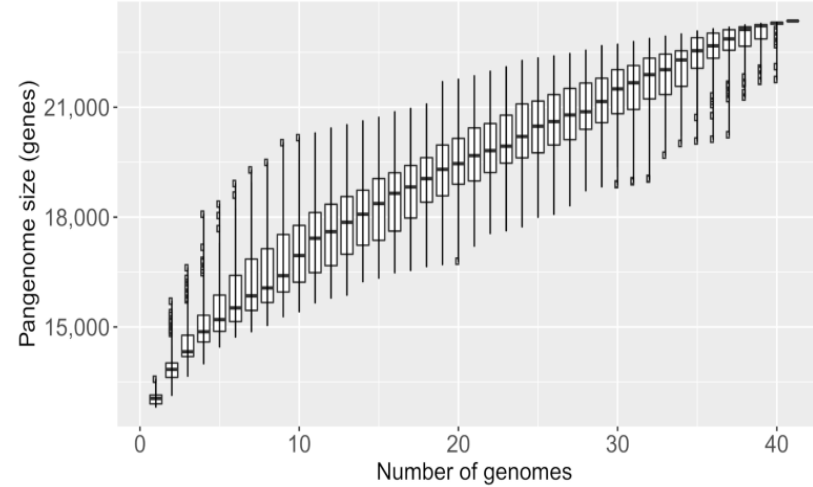
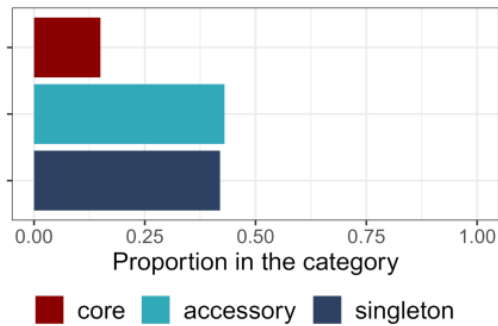
Pangenome



CAZyme








Effectors



Pre-print available on ResearchGate and bioRxiv

<https://www.biorxiv.org/content/10.1101/2022.03.07.483352v2>

## **Dissecting the *Pyrenophora tritici-repentis* (tan spot of wheat) pangenome**

 Ryan Gourlie,  Megan McDonald,  Mohamed Hafez,  Rodrigo Ortega-Polo,  Kristin E Low,  
 D Wade Abbott,  Stephen Strelkov,  Fouad Daayf,  Reem Aboukhaddour

doi: <https://doi.org/10.1101/2022.03.07.483352>

Let us know what you think on twitter!  
@GourlieRyan @McdonaldMeganc @hafez\_mnm  
@ropolo @sweetmicrobe @ReemWheat

# Acknowledgements

- Reem Aboukhaddour
- Megan McDonald
- Rodrigo Ortega-Polo
- Mohamed Hafez
- Stephen Strelkov
- Wade Abbott and Kristin Low
- Fouad Dyaaf
- HPC Biocluster Team
- Funders and Supporters



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