

# Insights into the tan spot genome and its effector genes

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Agriculture and  
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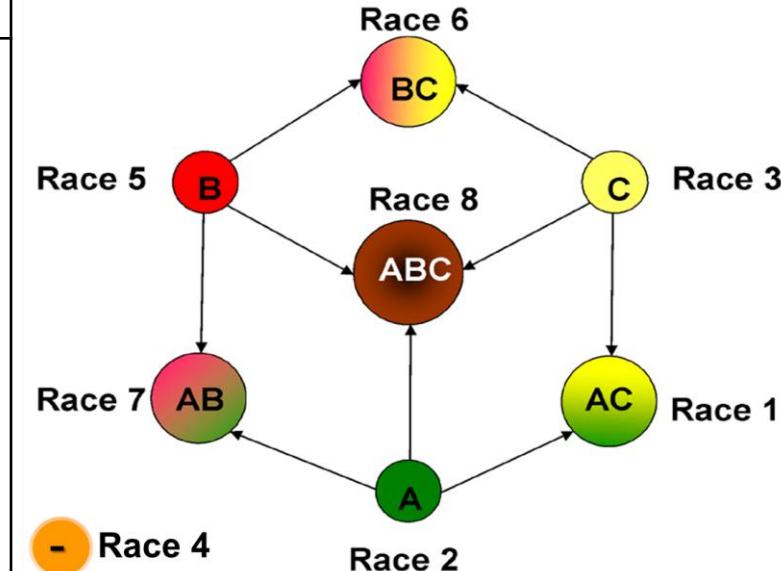
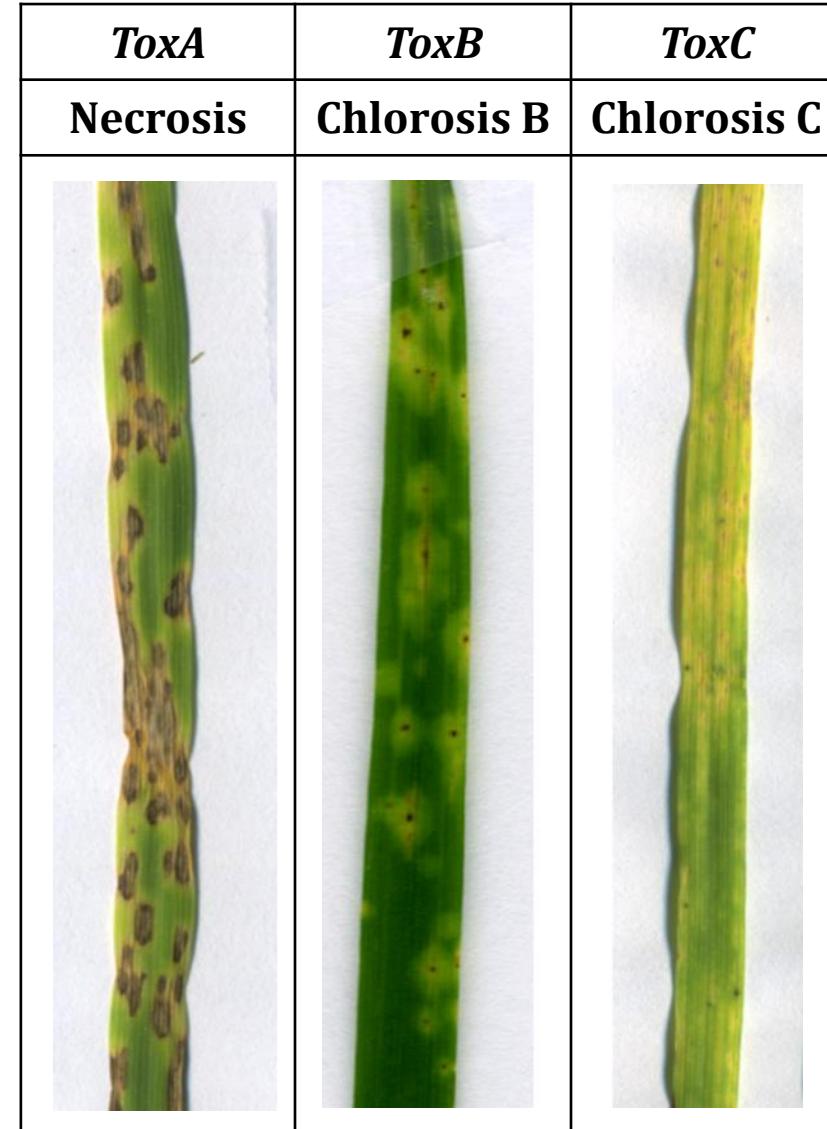
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# Tan spot of wheat

- *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>



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

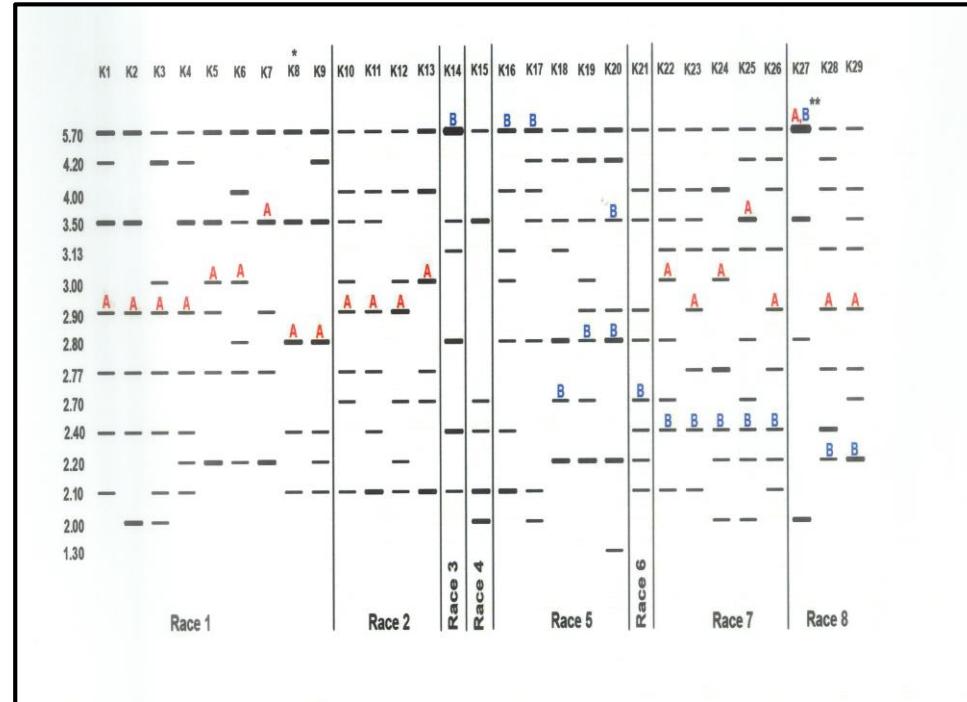
<sup>1</sup>Savary et al. 2019. Nat. Ecol. Evol. 3, 430-439

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

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

# Ptr genome

- Haploid ~35 to 40 Mb
- Primarily ToxA and ToxC producers have genomes sequenced
  - Recently many more released
  - 40 from this work 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**



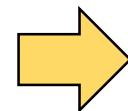
Aboukhaddour et al. 2009. Mol. Plant Pathol. 10, 201-212

# Objectives

- Sequence all races of *Ptr*
- Assemble pangenome
- Confirm movement of *ToxA* within the *Ptr* genome
- Identify how *ToxA* moved within *Ptr*
- Determine if *ToxB* could be mobile
- Explore evolution of *ToxB* within *Ptr* and other species

# Methods --- DNA & sequencing

Ptr grown in PDB flasks



DNA extraction



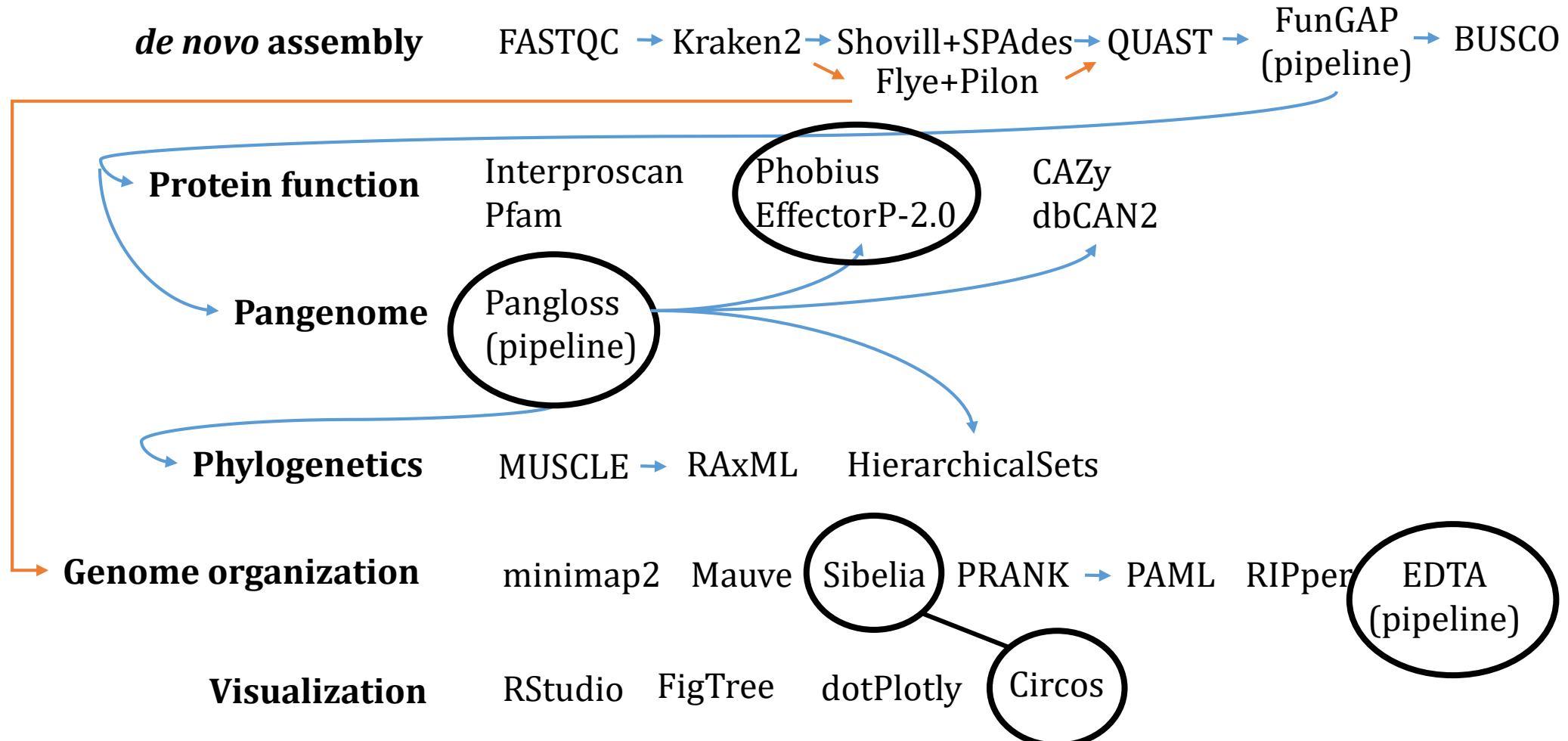
Race	ToxA	ToxB	ToxC	#sequenced
1	+	-	+	10
2	+	-	-	6
3*	-	-	+	6
4	-	-	-	3
5	-	+	-	7
6	-	+	+	1
7	+	+	-	3
8*	+	+	+	3
novel	-	+	-	1
			Total	40

Short-reads Illumina HiSeq

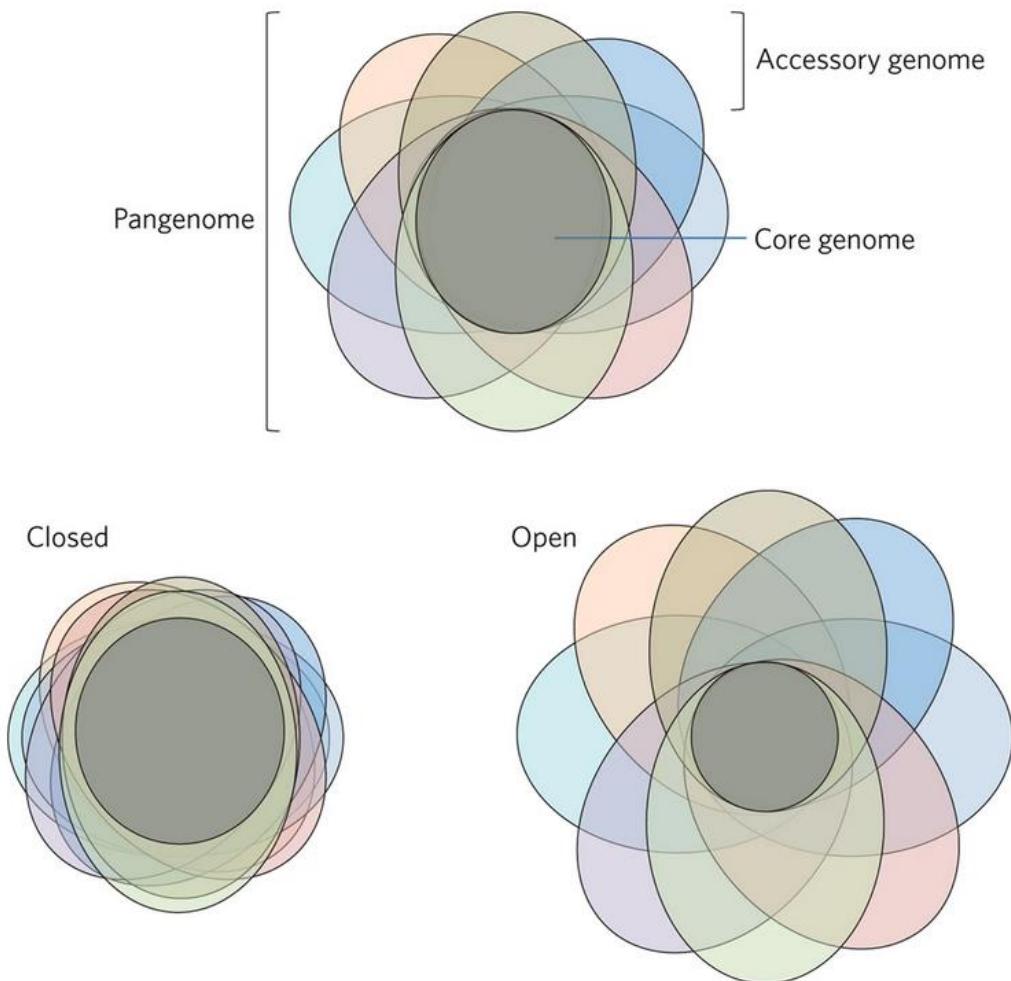


Long-reads PacBio RS II  
race 8 and 3 @ Genome Quebec

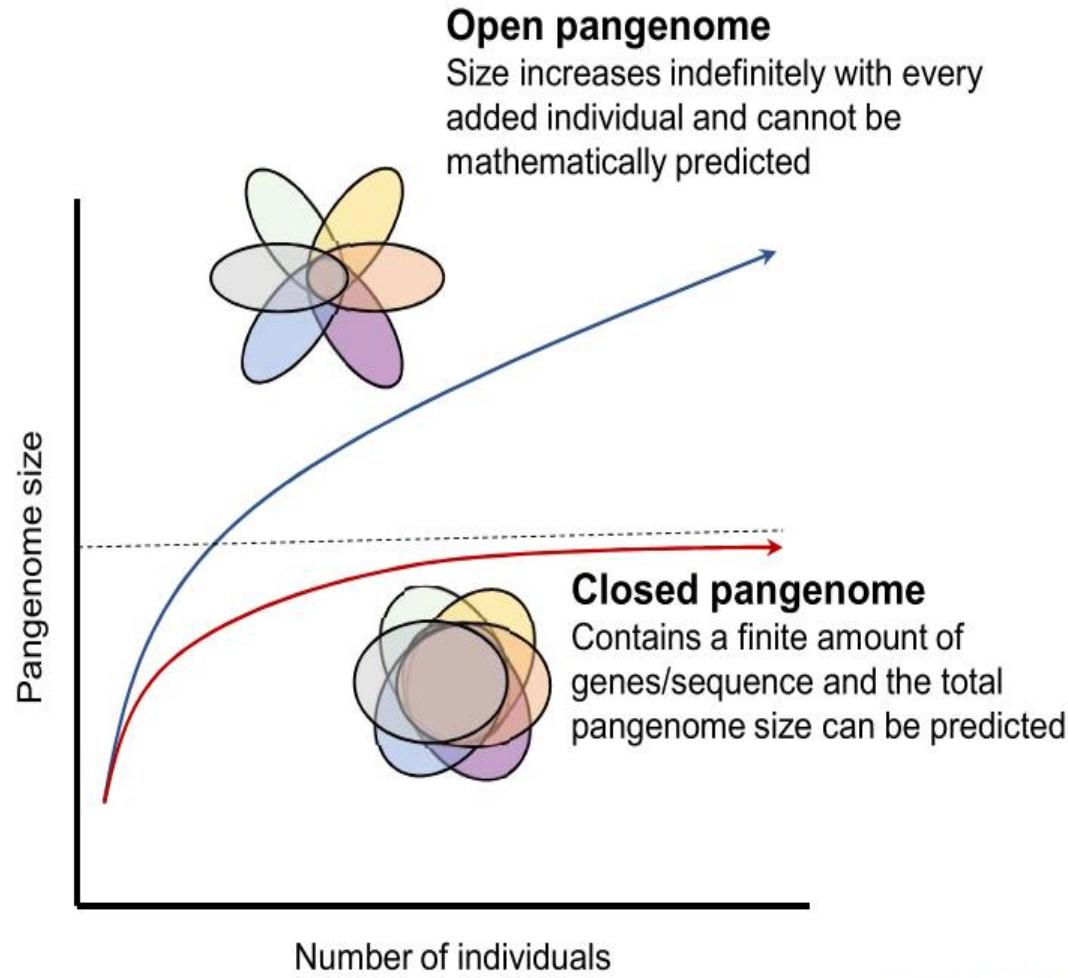
# Methods -- Pipeline & Programs



# Pangenomes

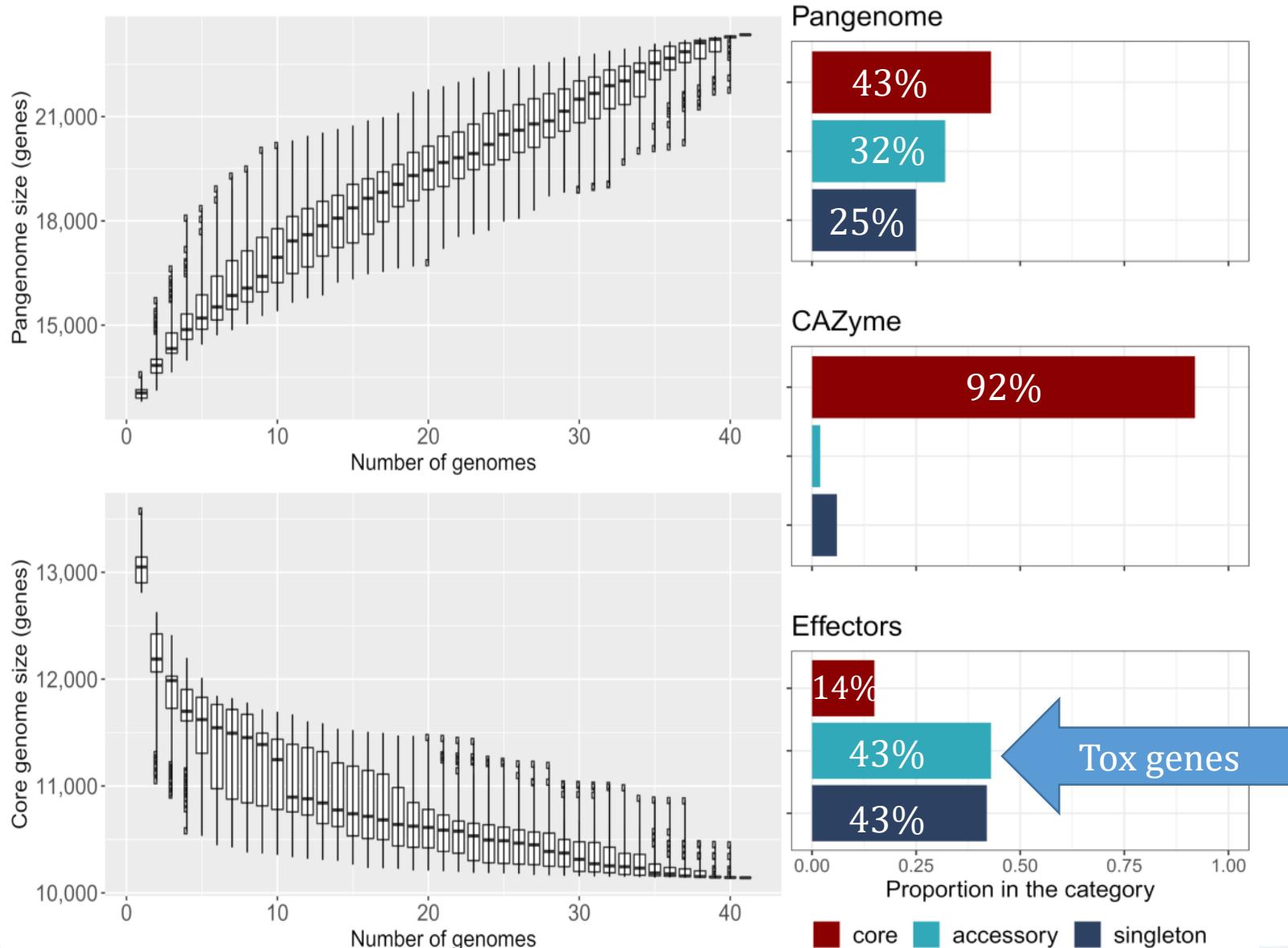


*Nat Microbio.* **2**, 17040 (2017)



*Trends in Gen.* **36**, 132-145 (2019)

# Ptr pangenome



# 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 (**r**epeat **i**nduced **p**oint-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)



Barbara McClintock; Nobel Prize 1983

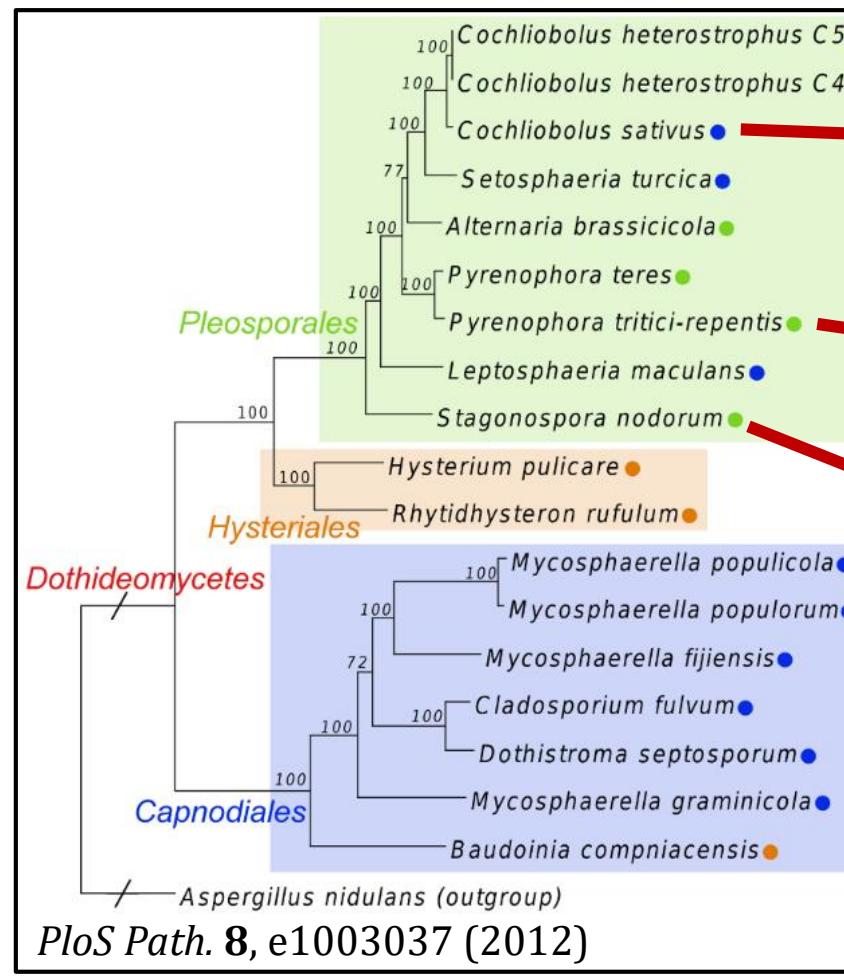
## How TEs are classified

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

# 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 blotch

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



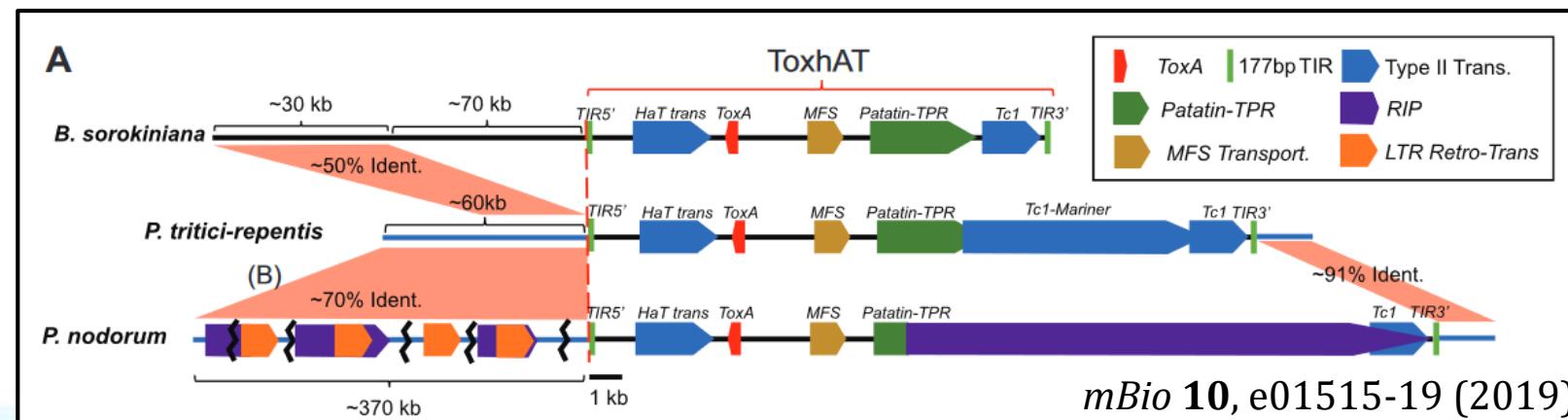
## Tan spot

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

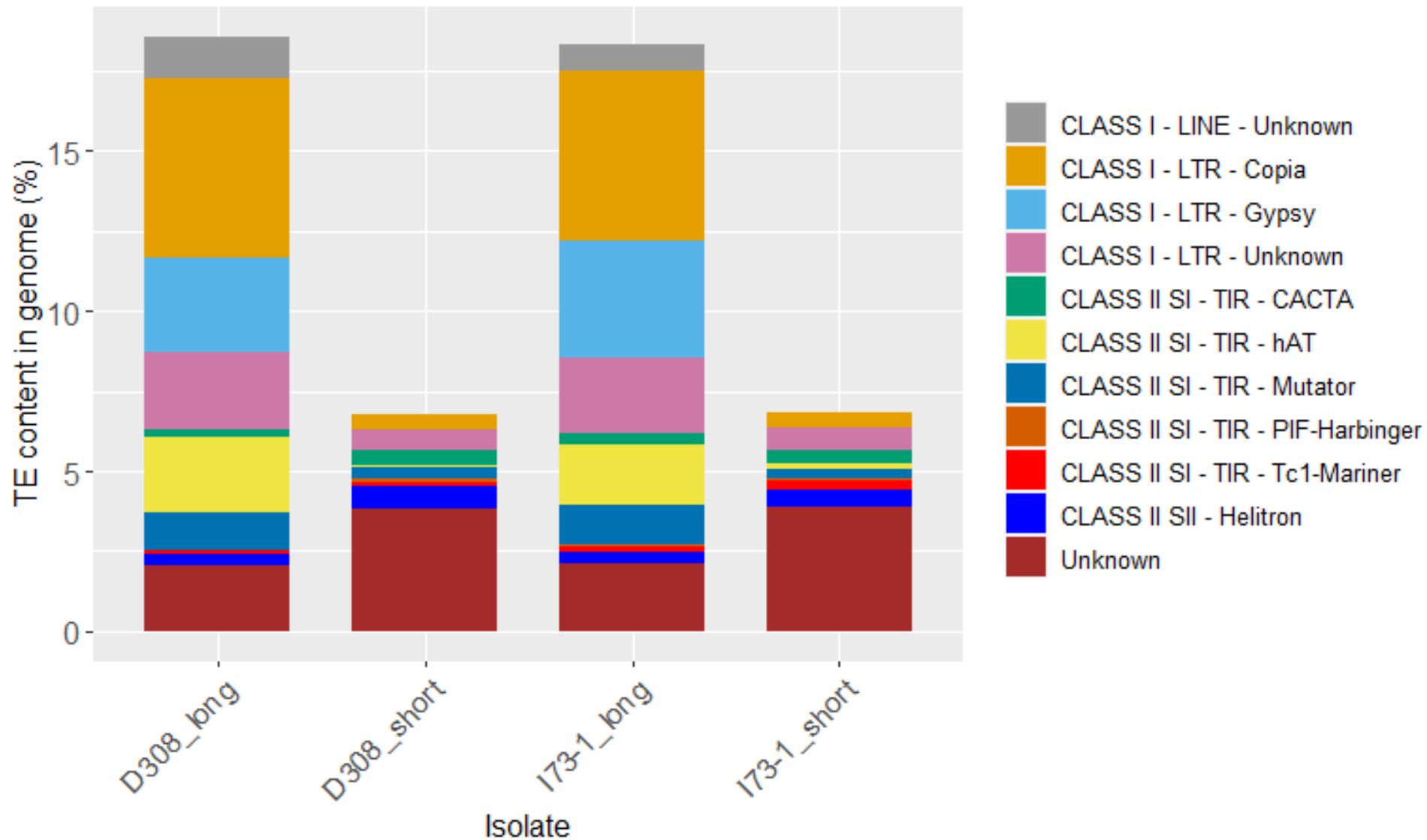


## Septoria blotch

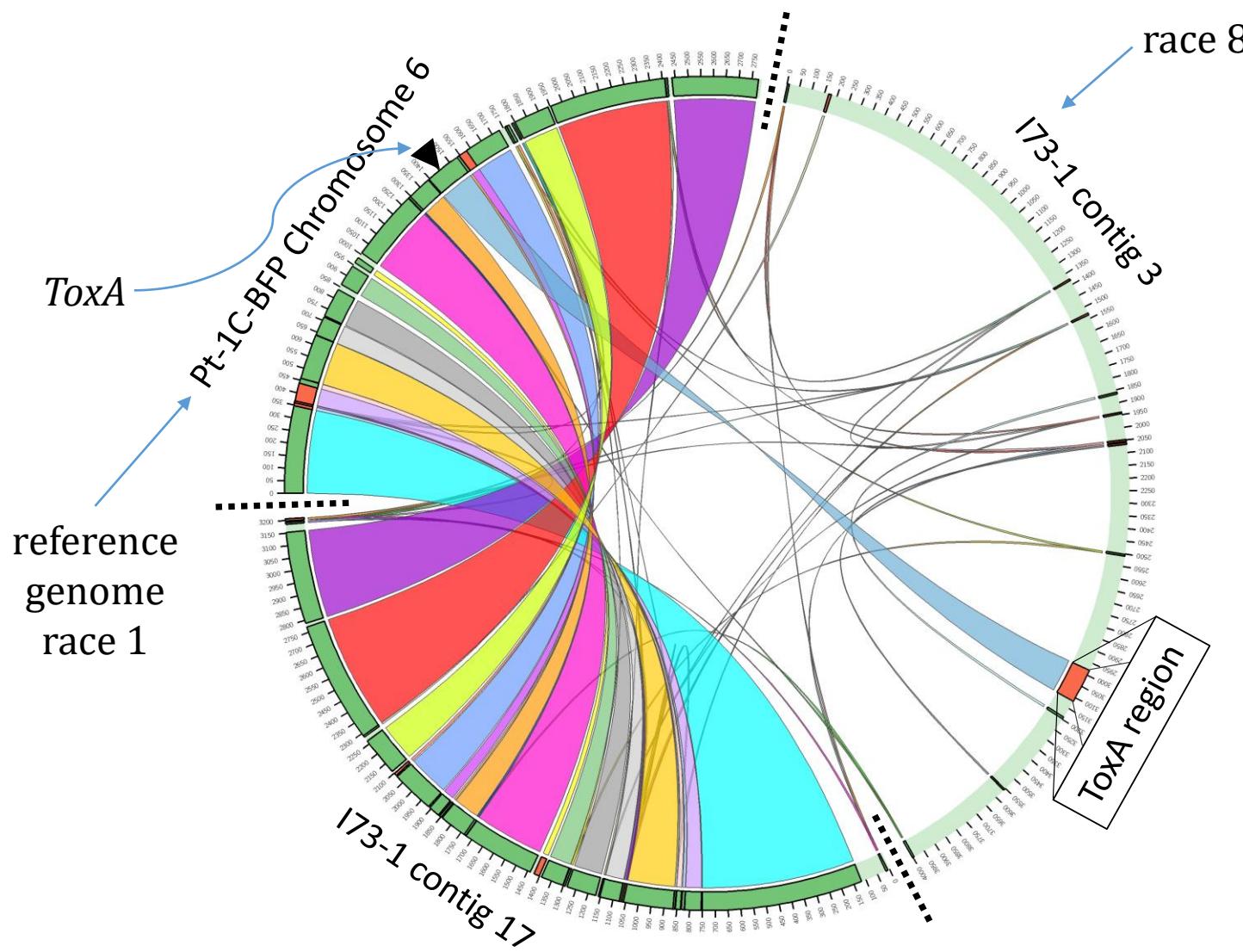
*Nat Genet.* **38**, 953-956 (2006)



# Transposable element content



# Intraspecific *ToxA* translocation

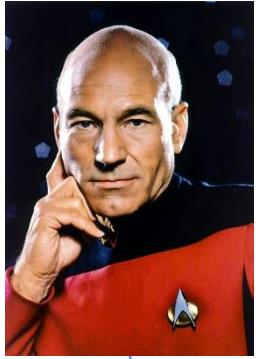


- Confirms work with PFGE<sup>1</sup>
- 143 kb element
- ToxhAT fully nested
- Nested transposons associated with rapid evolution<sup>2</sup>

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

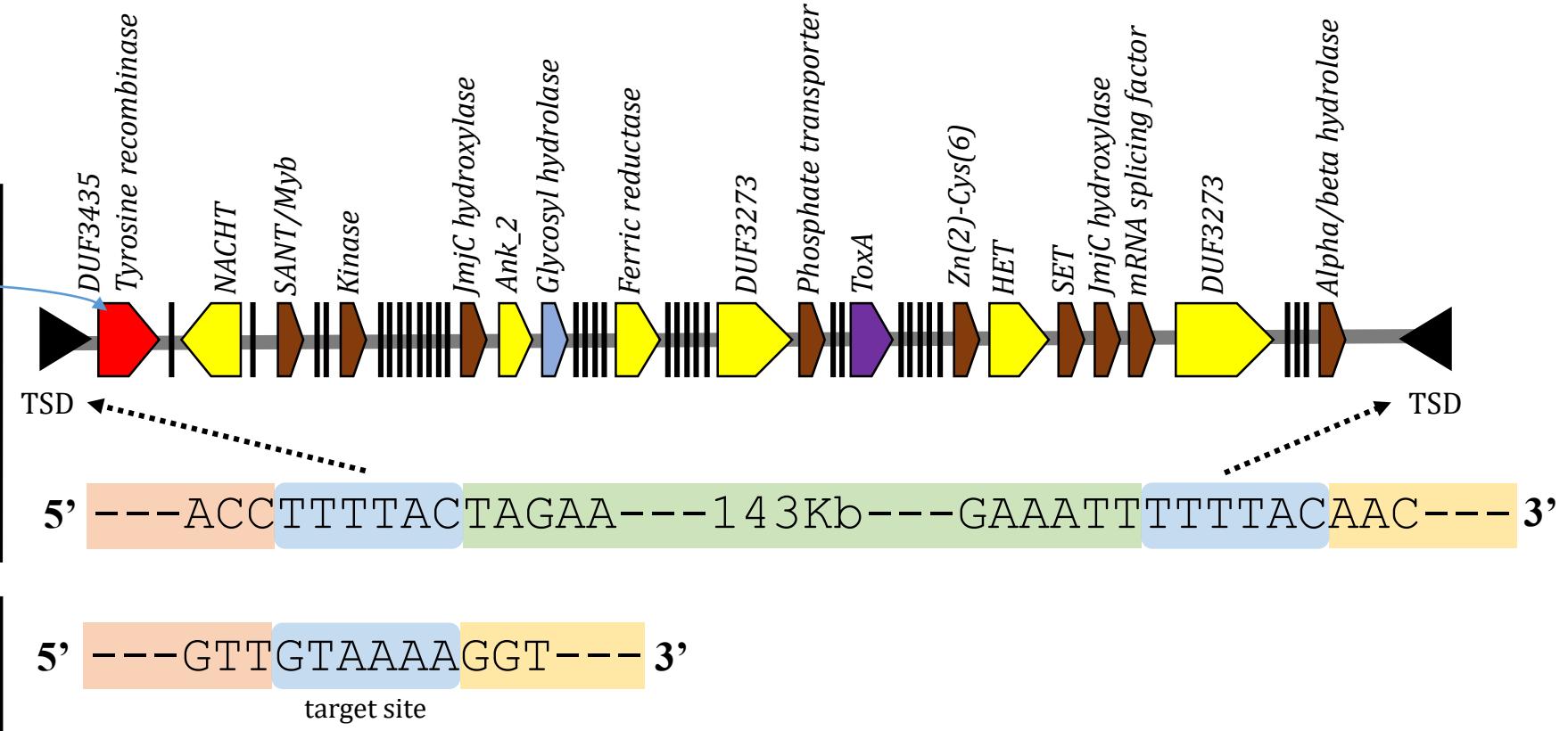
<sup>2</sup>Mol. Sciences. **20**, 3597 (2019)

# ToxA Starship ‘Horizon’ schematic



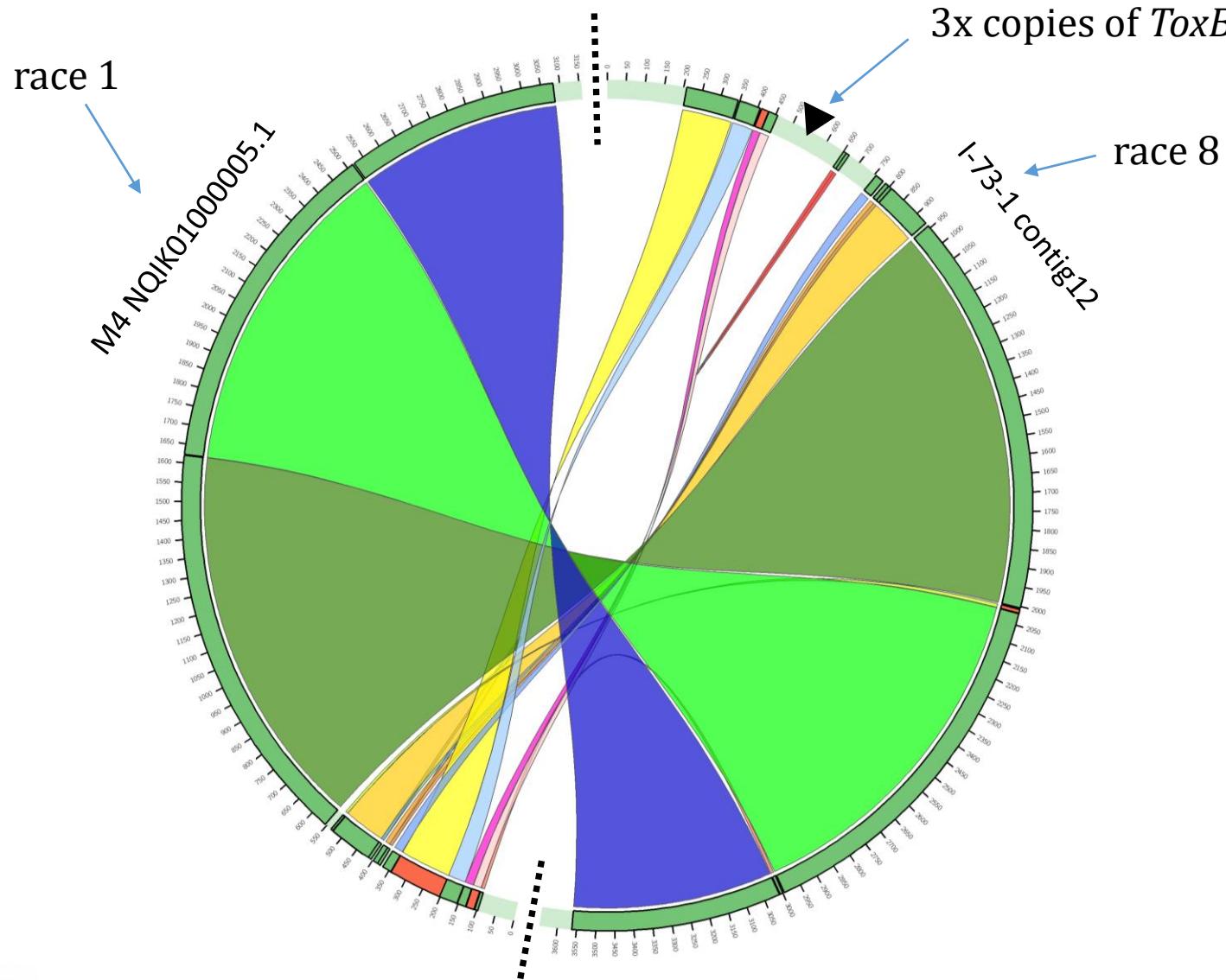
I-73-1 contig 3

Pt-1C-BFP chr 1



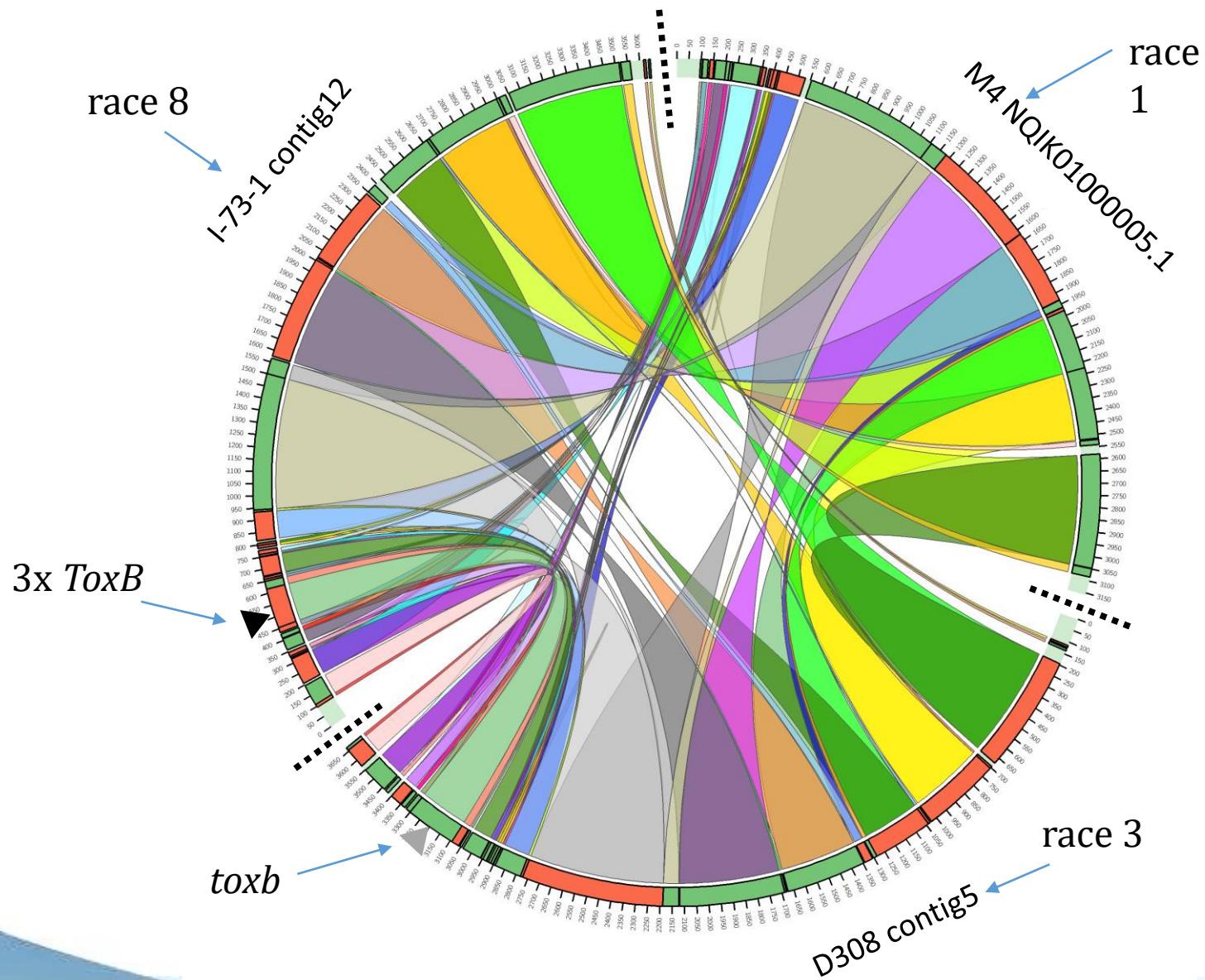
Red = Transposase  
 Yellow = Starship cargo  
 Purple = Tox gene  
 Blue = CAzyme  
 Brown = annotated  
 Black bar = unknown

# *ToxB* on a putative transposon



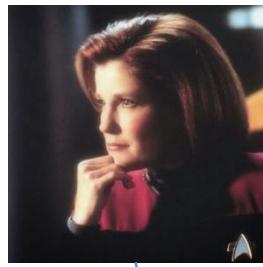
- 294 Kb element
- Small 12 Kb region contains 3x copies of *ToxB*
- Aligns nowhere in genome of race 1 isolates
- Edges appear to be **terminal inverted repeats**
- Target site visible in M4 race 1

# *ToxB* on a putative transposon



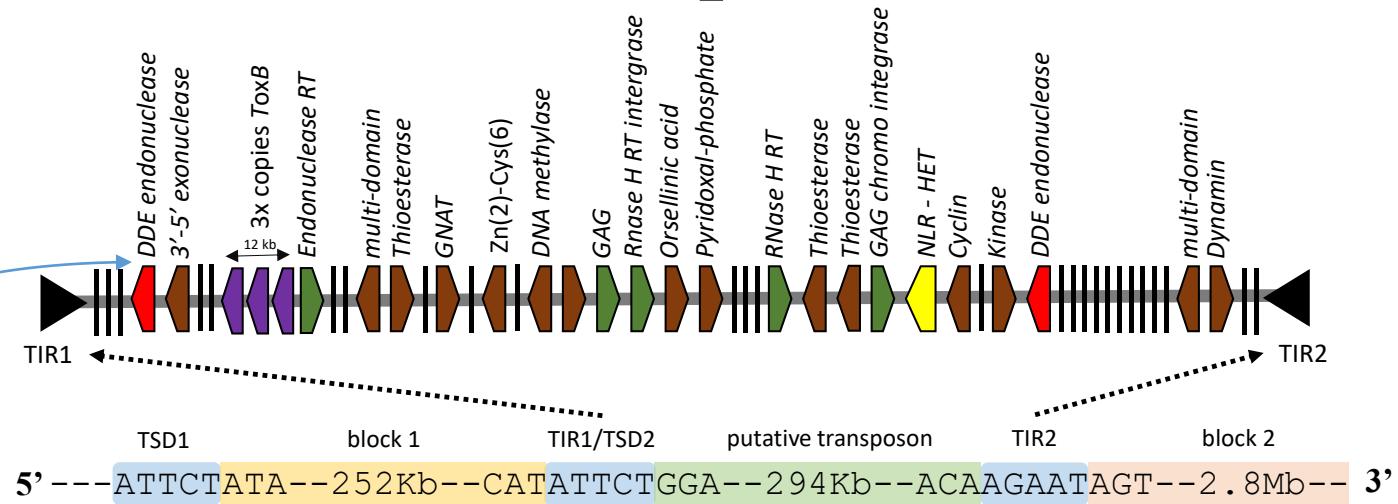
- Same element present in race 3 with *toxb*  
*(actually a bit larger)*
- *toxb* in same general area but as single copy

# ToxB on putative Starship 'Icarus'

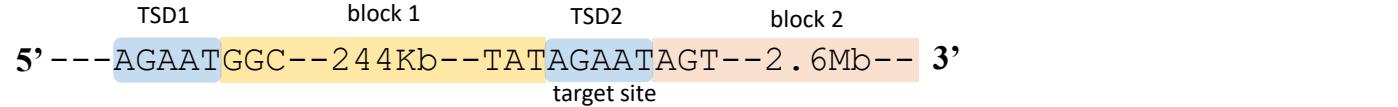


?

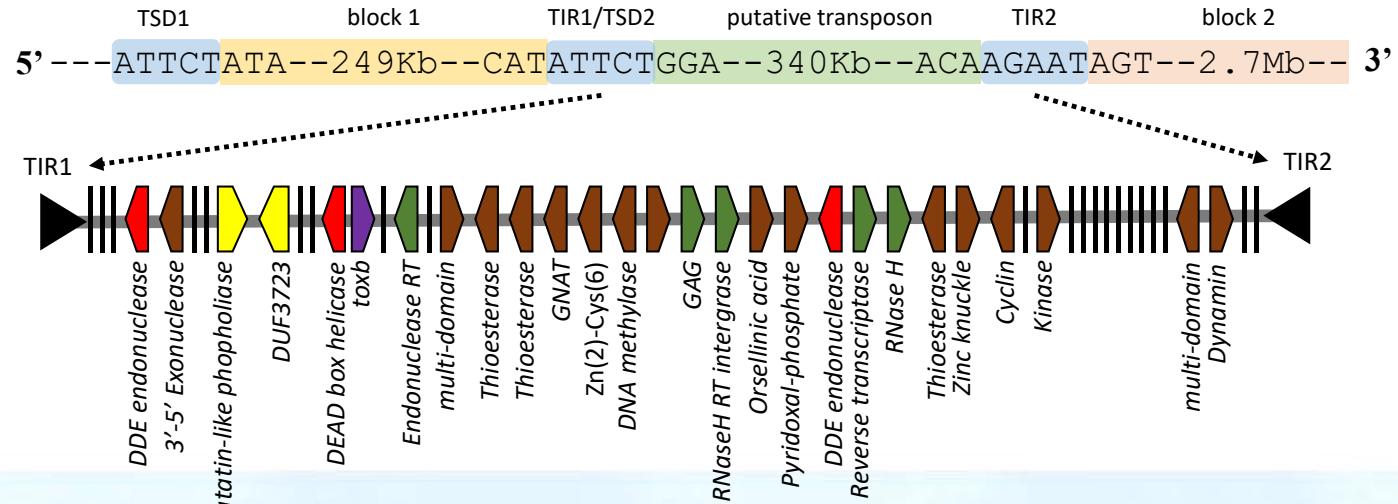
I-73-1 contig 12



M4 NQIK01000005



D308 contig 5



Red = Transposase  
Yellow = Starship cargo  
Purple = Tox gene  
Brown = annotated  
Black bar = unknown  
Green = Class I RNA TE

# **Sequence analysis and structural features of the ToxB gene in *Pyrenophora tritici-repentis* and other ascomycete species**

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<sup>c</sup>Centre for Crop and Disease Management, Department of Environment and Agriculture, Curtin University, Bentley, WA, Australia



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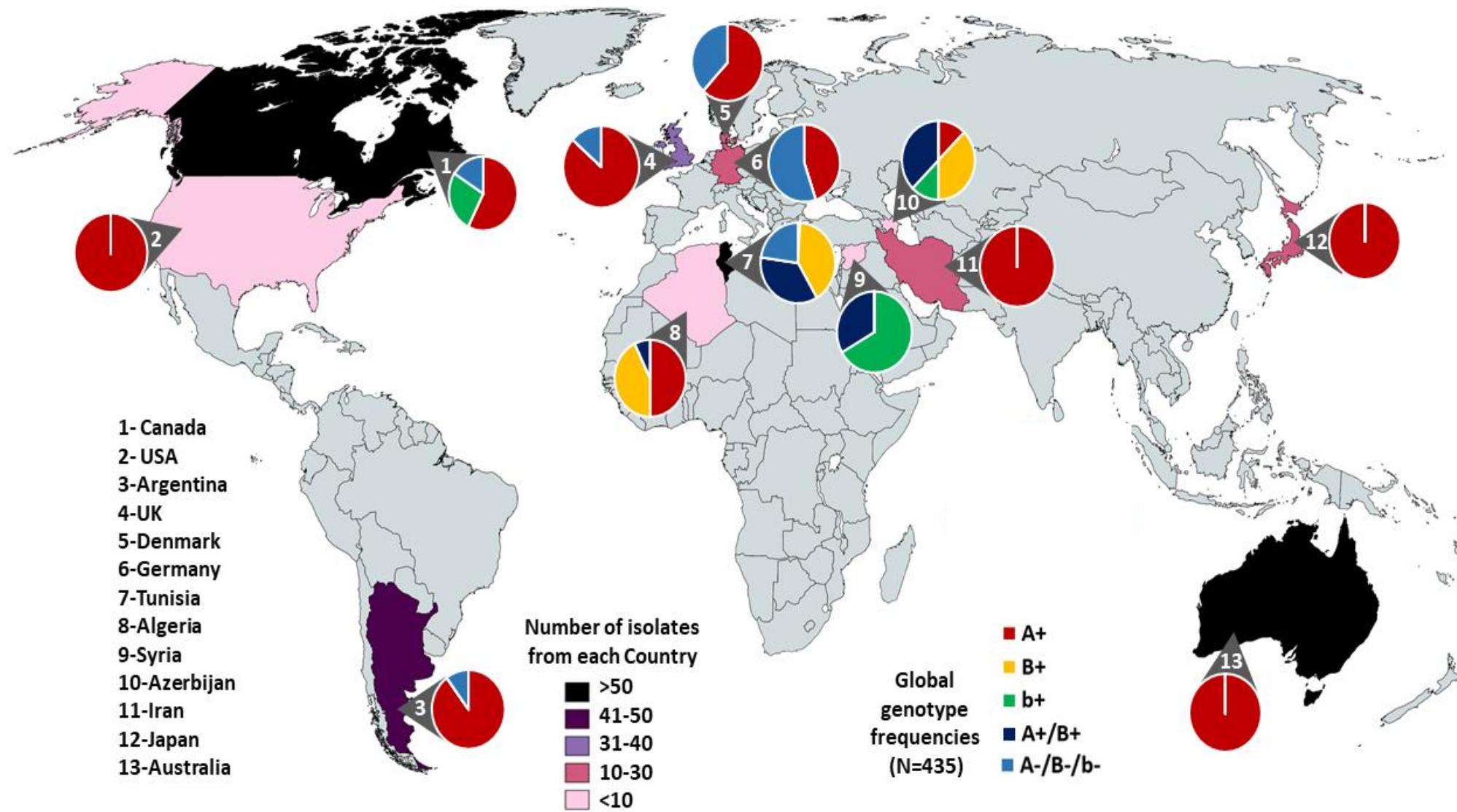


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# *ToxA/ToxB/toxb* distribution

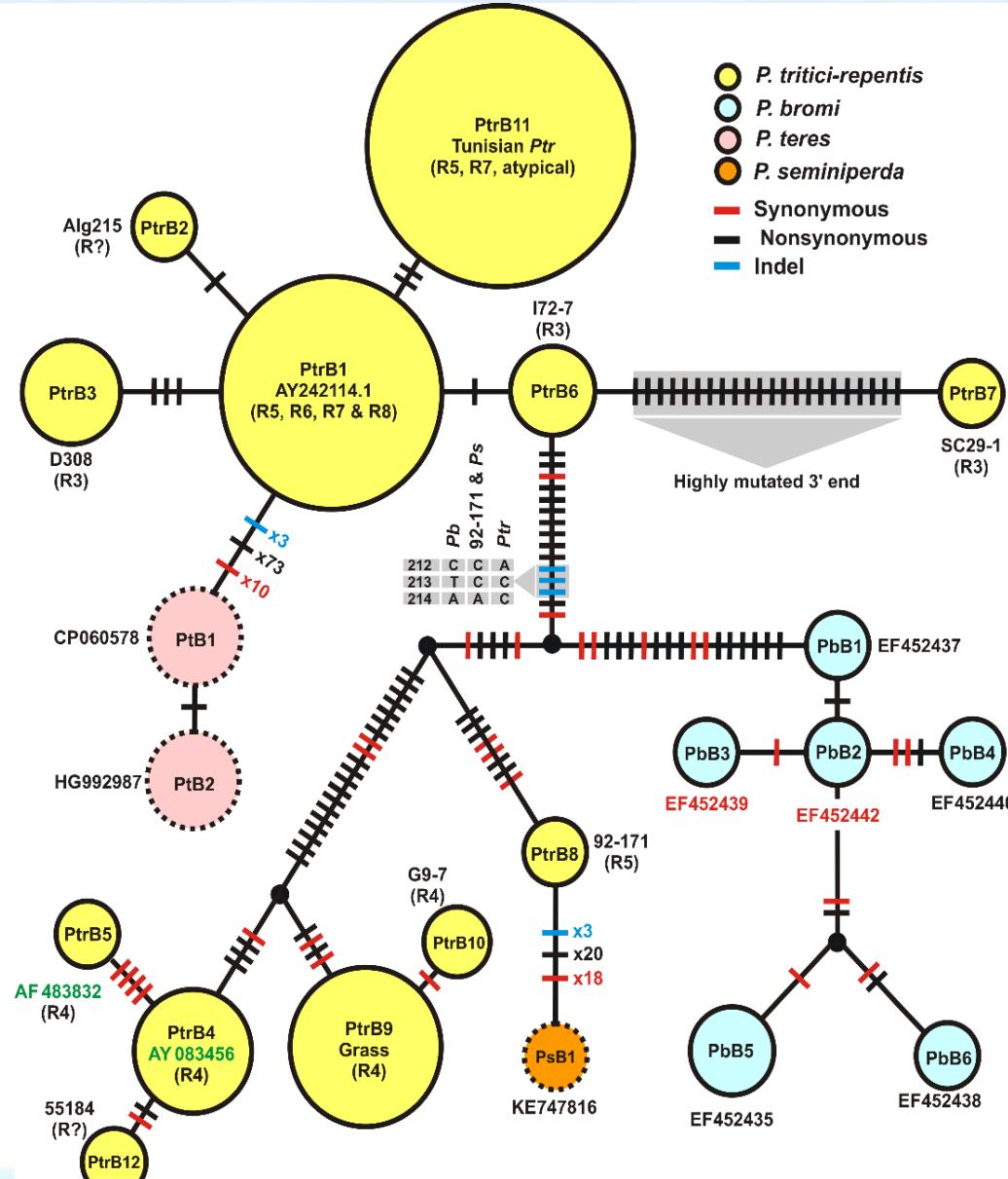


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# ToxB haplotype network

- 161 ORF used for this network (GenBank & PCR)
- PopArt generated *ToxB* haplotype network
- Translation of *ToxB* to protein revealed prevalence of **non-synonymous** over **synonymous** mutations
- **21 *ToxB* haplotypes**
  - *P. tritici-repentis* (12)
  - *P. bromi* (6)
  - *P. teres* (2)
  - *P. seminiperda* (1)



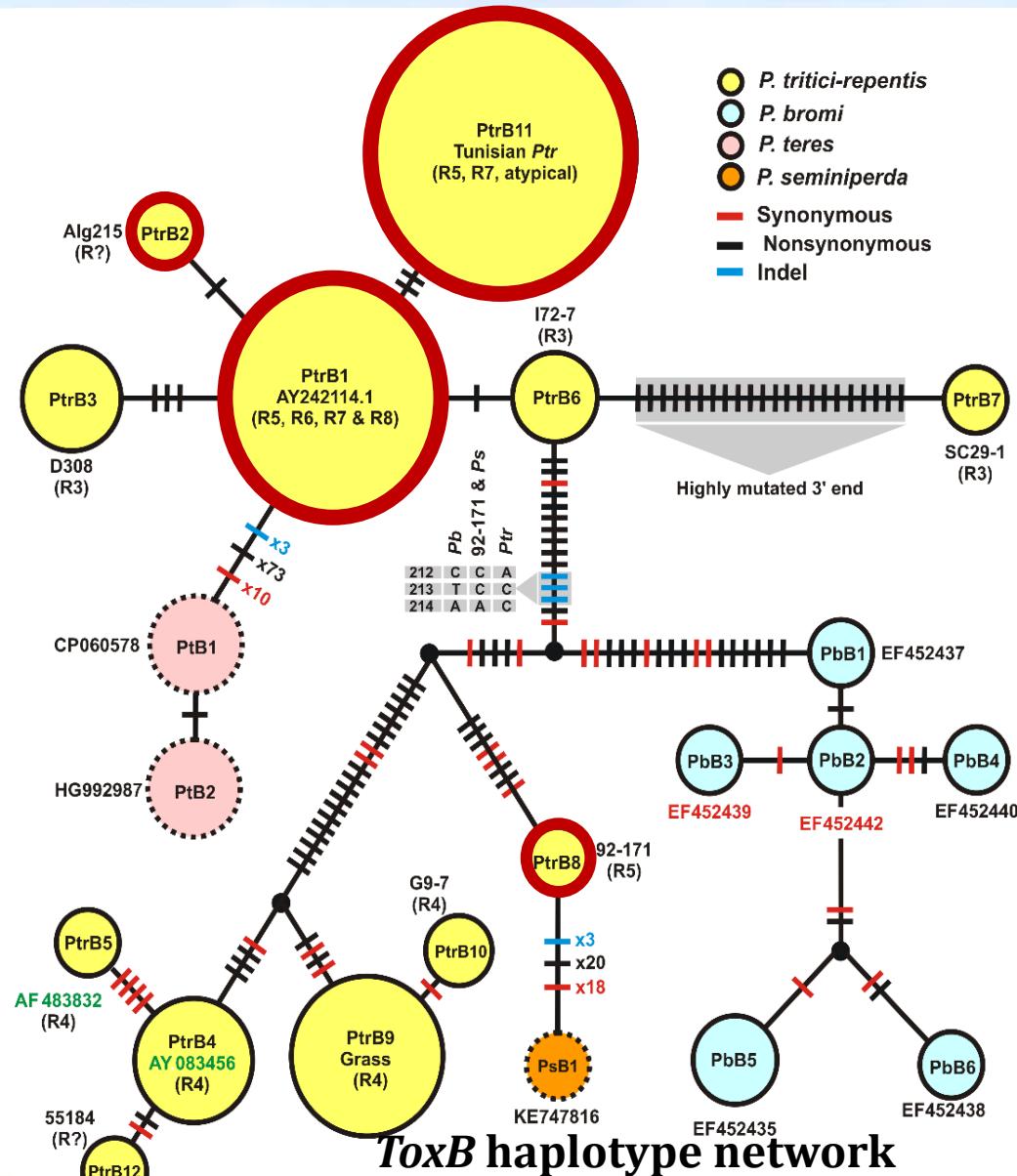
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# ToxB active isoforms



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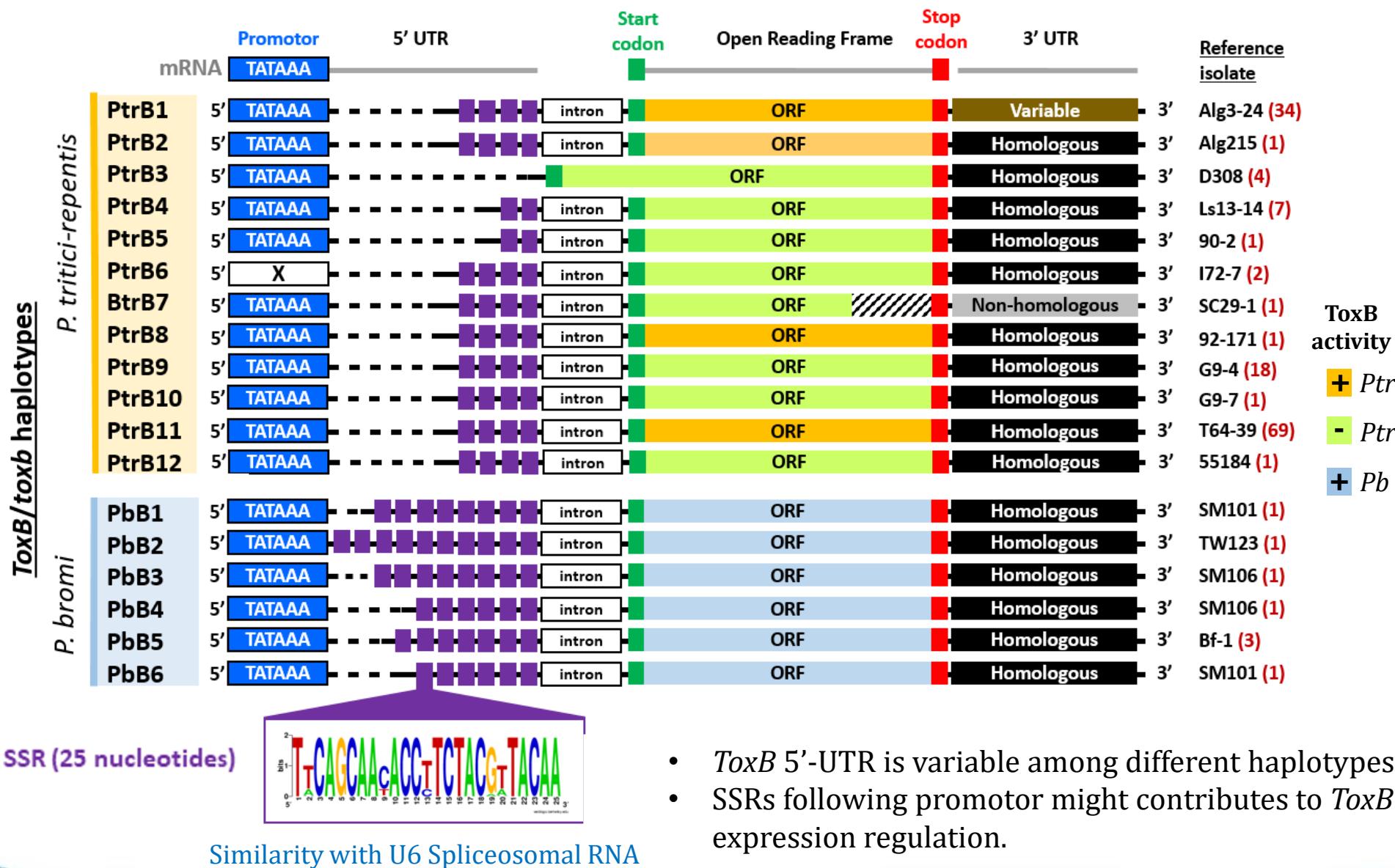
- Only 4 active isoforms (**red rims**)
- Despite high similarity, homologs are inactive (*toxb*)
- Lack of chlorosis-inducing activity by race 3 and 4 isolates that contain *toxb* homologs
  - Altered signal peptide
  - Altered protein folding
  - Insufficient copy number
  - Transcription deficiency



# ToxB flanking sequences



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- ToxB 5'-UTR is variable among different haplotypes.
- SSRs following promotor might contributes to ToxB expression regulation.

# ToxB in Ascomycetes

Previously ToxB identified in  
**(Dothidiomycetes)**

Ptr  
*P. bromi*  
*Pyrenophora teres*  
*Cochliobolus sativus*  
*Alternaria alternata*  
*Pyrenophora graminea*

**(Sordariomycetes)**

*Colletotrichum fioriniae*  
*Colletotrichum gloeosporioides*  
*Magnaporthe oryzae*  
*Macrophomina phaseolina*

Martinez et al., 2004; Strelkov et al., 2006;  
 Andrie et al. 2008; Nyarko et al. 2014:

Species	Host	Disease	Our work
<i>Bipolaris oryzae</i>	Rice	Brown spot	New
<i>Botryosphaeria dothidea</i>	Broad range	Cankers	
<i>Colletotrichum fioriniae</i>	Broad range	Anthracnose	
<i>Colletotrichum gloeosporioides</i>	Broad range	Bitter rot	
<i>Colletotrichum karsti</i>	Broad range	Anthracnose	New
<i>Colletotrichum nymphaeae</i>	Broad range	Anthracnose	New
<i>Colletotrichum orbiculare</i>	Melons	Anthracnose	New
<i>Colletotrichum salicis</i>	Broad range	Anthracnose	New
<i>Colletotrichum scovillei</i>	Pepper	Anthracnose	New
<i>Colletotrichum sidae</i>	Mallows	Anthracnose	New
<i>Colletotrichum simmondsii</i>	Broad range	Anthracnose	New
<i>Colletotrichum spinosum</i>	Broad range	Anthracnose	New
<i>Diaporthe batatas</i>	Sweetpotato	Dry rot	New
<i>Diaporthe citri</i>	Citrus	Rot diseases	New
<i>Diplodia corticola</i>	Oak	Bot canker	New
<i>Hyaloscypha bicolor</i>	Broad range	Endophyte	New
<i>Lasiodiplodia theobromae</i>	Broad range	Rotting and dieback	New
<i>Leptosphaeria biglobosa</i>	Canola	Stem canker	New
<i>Macrophomina phaseolina</i>	Broad range	Damping off and rot	
<i>Pyrenophora bromi</i>	Bromgrass	Leaf spot	
<i>Pyrenophora seminiperda</i>	Grass	Leaf spot	New
<i>Pyrenophora teres f. maculata</i>	Barley	Net blotch	
<i>Pyrenophora tritici-repentis</i>	Wheat	Tan spot	
<i>Pyricularia pennisetigena</i>	Broad range	Leaf blight	New



Mohamed Hafez

Leotiomycetes

# Conclusions and future work

- First long-read assemblies of race 3 and race 8
- 40 new short-read assemblies of all races
- Ptr has an open-pangenome and is highly adaptable
- *ToxA* is nested within multiple transposons
- ToxhAT and novel Starship ‘Horizon’
- *ToxB* present on different Starship (possibly disabled) ‘Icarus’
- First *ToxB* haplotype network and flanking region investigation

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>

Our lab is looking for PhD students!

Contact Dr. Reem Aboukhaddour for details ☺

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# Acknowledgements

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- Mohamed Hafez
- Stephen Strelkov
- Wade Abbott and Kristin Low
- Fouad Dyaaf
- HPC Biocluster Team
- Funders and Supporters



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