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

Gourlie *et al.*, 2022. bioRxiv. <https://doi.org/10.1101/2022.03.07.483352>

# Outline

- Introduce *Pyrenophora tritici-repentis*
- Overview of our pangenome study
  - open pangenome and adaptability
  - phylogeny in relation to effectors and geography
  - effector gene mobility on large transposons
- Conclusions

# Tan spot of wheat

- *Pyrenophora tritici-repentis* (Ptr)
- Isolated from *Agropyron repens* in early 1900s - “weakly pathogenic” on grasses
- Emergence on wheat in the 1940s via HGT<sup>1,2</sup>
  - 14 kb transposon ToxhAT
- Today worldwide occurrence on wheat  
~5% global losses<sup>3</sup>


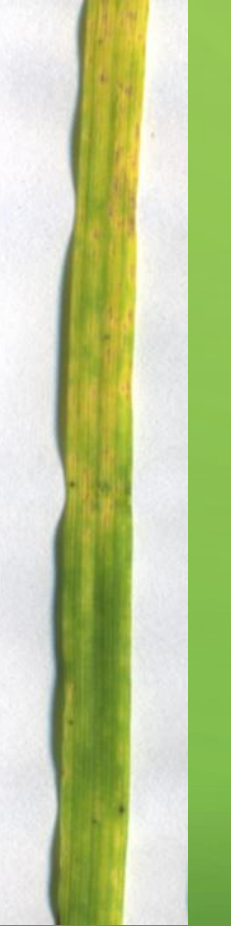


<sup>1</sup>Nat. Genet. **38**, 953-956 (2006)

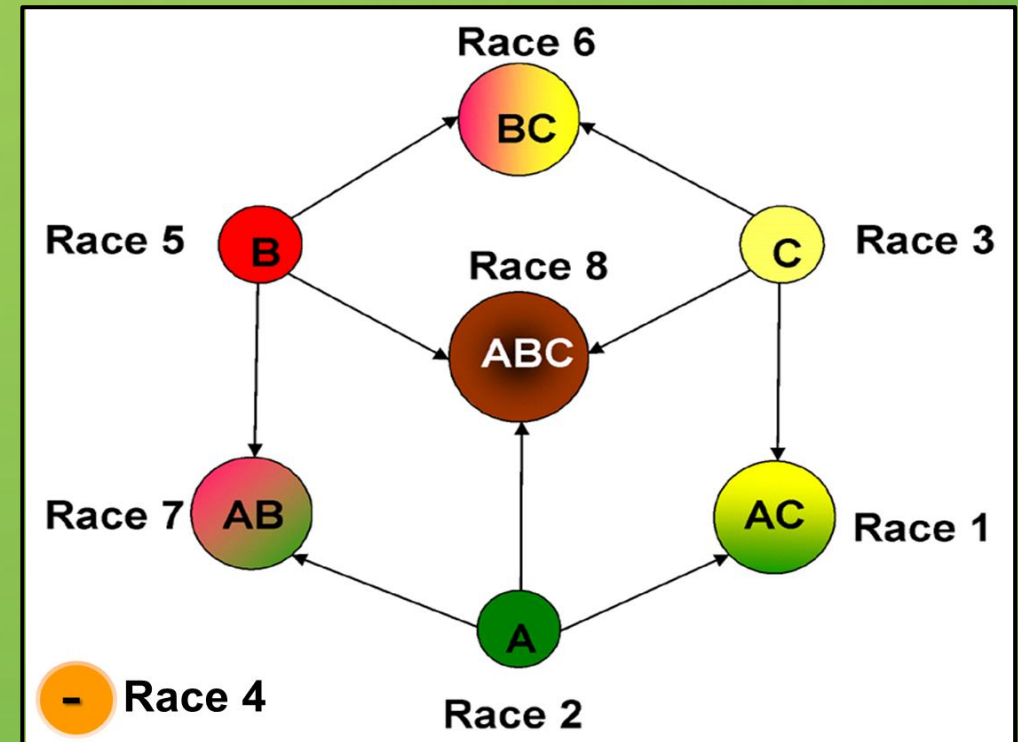
<sup>2</sup>mBio **10**, e01515-19 (2018)

<sup>3</sup>Nat. Ecol. Evol. **3**, 430-439 (2019)

# Ptr and its necrotrophic effectors

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

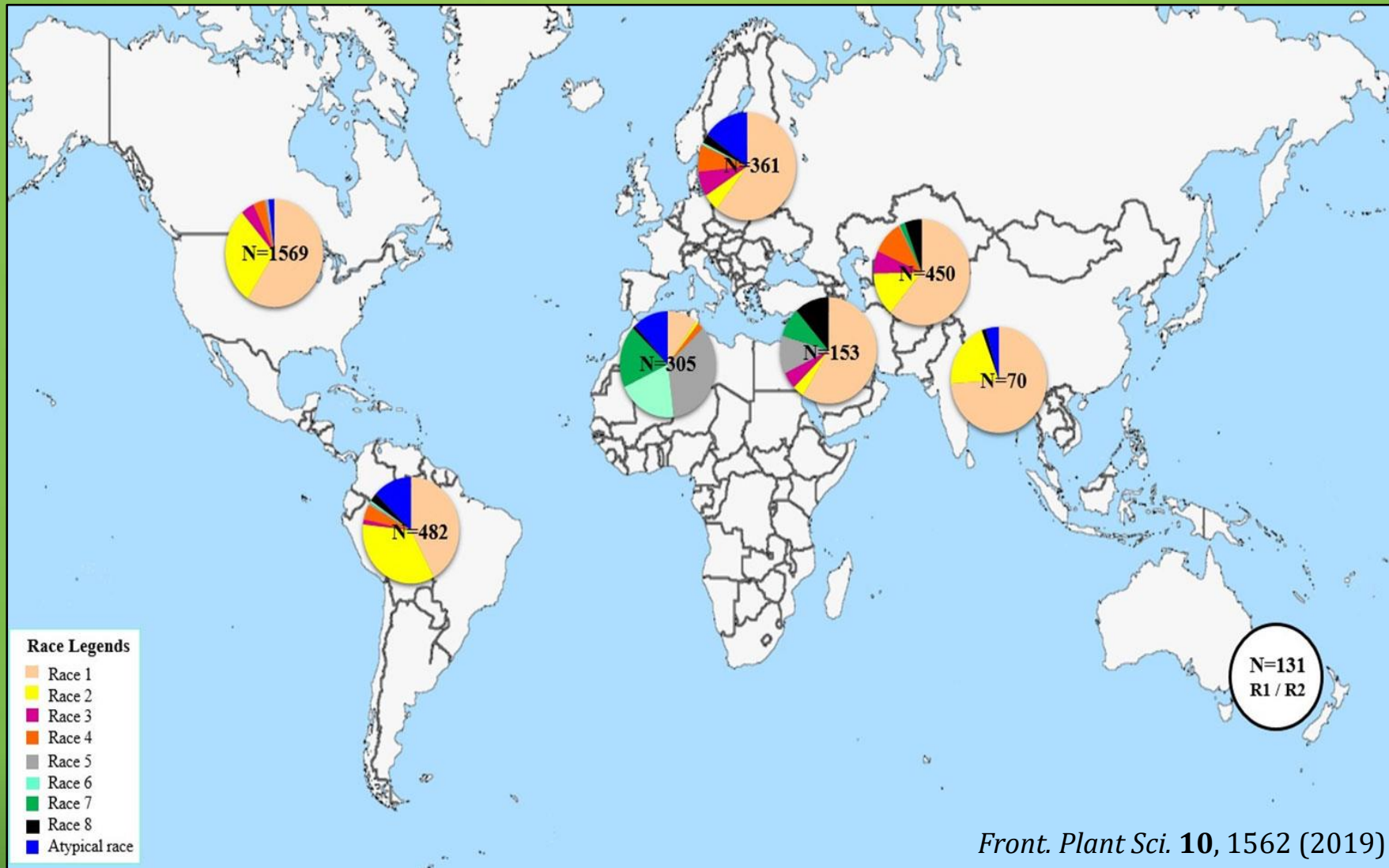
Ptr race structure



CJPP 25, 339-349 (2003)



# Ptr and its necrotrophic effectors

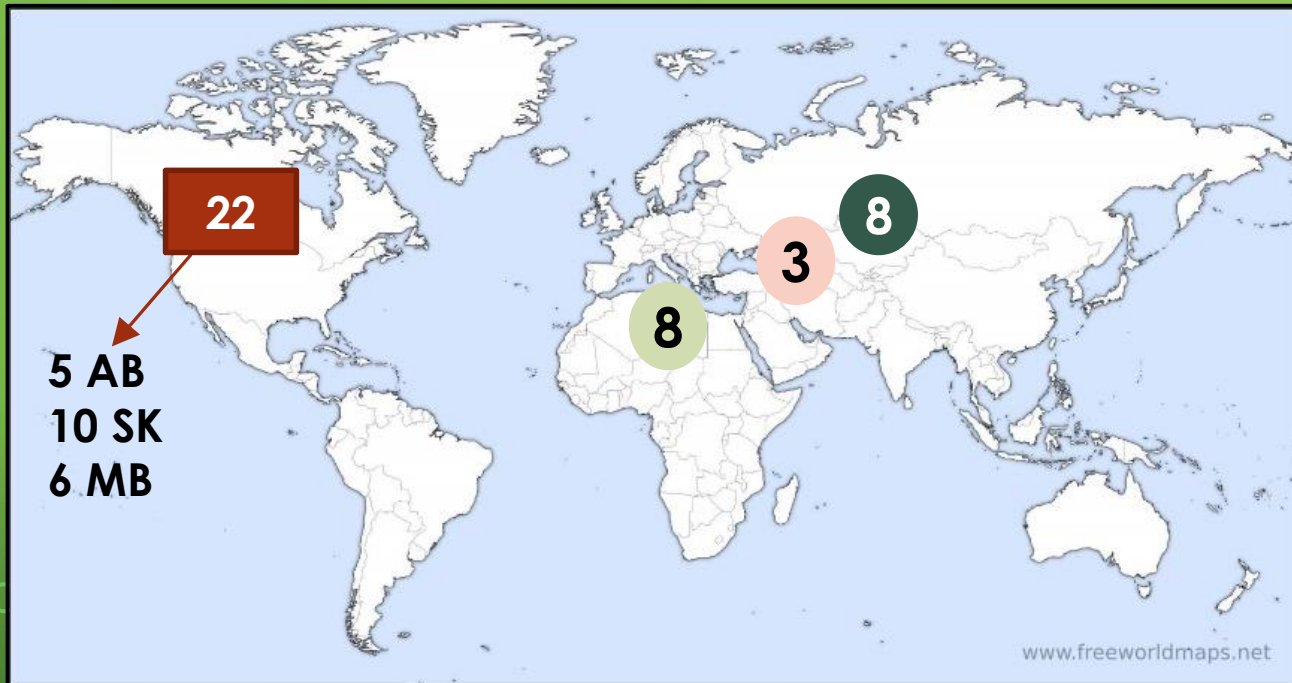


# Objective

- Sequence all Ptr races and from the centre of wheat origin
  - Previous emphasis on North America and Australian isolates
- Provide insights into the evolution of the species as a whole
  - How is Ptr so adaptable?
  - How plastic is the Ptr genome?
- Add to understanding of effector evolution and mobility
  - Movement of *ToxA* within Ptr (previous PFGE work)
  - Is *ToxB* mobile like *ToxA*?

# Sequenced isolates

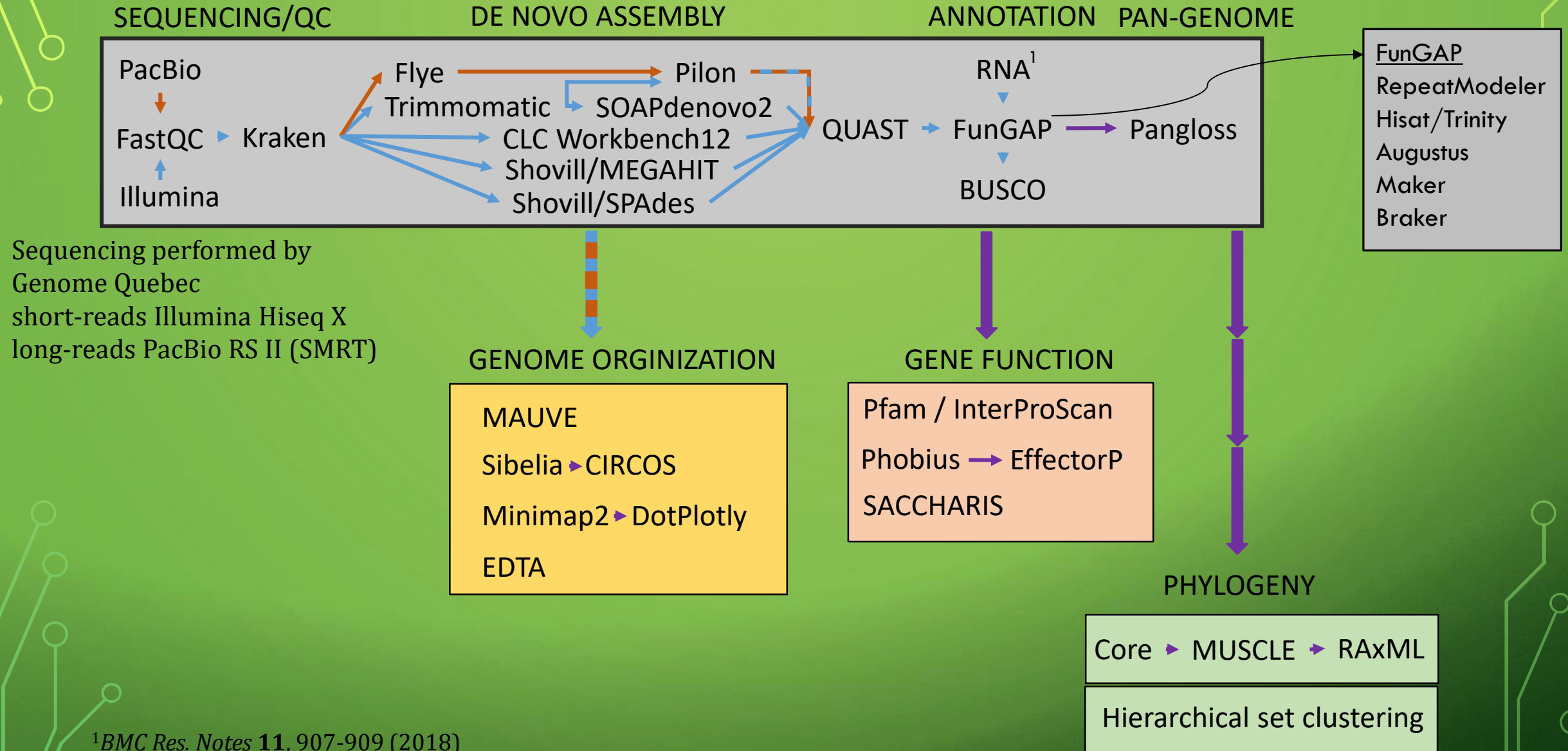
- Collection dates range from 1990 to 2017
- Grown on ¼ conc. PDB for ~7 days @ ~25°C
- Fungal mats washed and freeze-dried
- DNA extracted w/ 'Genomic-tip 20/G' or 'Genomic-tip 100/G' (Qiagen) for long-reads



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

\*sequenced with long-reads as well

# Ptr genomics pipeline



<sup>1</sup>BMC Res. Notes **11**, 907-909 (2018)



# Assembly details

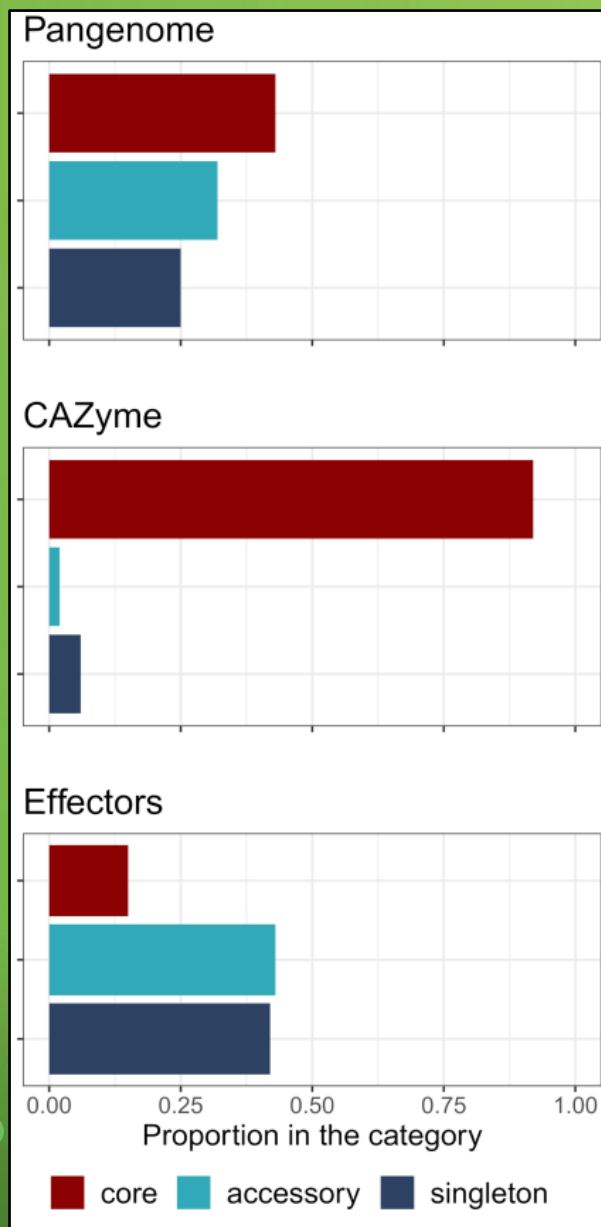
- Assembly completeness BUSCO >99%
- Short-read genome size –  $34.8 \pm 2.1$  Mb
  - TE content ~7% of genome
- Long-read genome size –  $39.8 \pm 1$  Mb
  - >150% more TEs
- Non-pathogenic genomes ~2 Mb larger than pathogenic genomes
  - Expansion of non-pathogenic genomes via TE

**Table 1.** Summary statistics for 40 isolates of *Pyrenophora tritici-repentis* sequenced with Illumina HiSeq X and assembled with Shovill/SPAdes, and two isolates sequenced with PacBio and assembled with Flye/Pilon (table is sorted by race then isolate name).

Isolate	Race	NE <sup>1</sup>	Year	Location <sup>2</sup>	Host	Size (Mb)	Contigs	GC (%)	TE (%)	N50 (kbp)	Genes	BUSCO (%)	Accessory (%)
ASC1	1	AC	1985	Manitoba	<i>T. aestivum</i>	34.78	6,495	50.9	6.9	65.5	13,124	99.3	22.5
I-33-1	1	AC	2001	Azerbaijan	<i>T. aestivum</i>	35.06	6,850	50.9	6.9	75.2	13,055	99.5	22.1
L3-1	1	AC	2016	Alberta	<i>T. durum</i>	34.93	6,666	50.9	6.9	77.2	13,115	99.4	22.4
L4-1	1	AC	2016	Alberta	<i>T. durum</i>	34.74	6,361	50.9	6.9	72.4	13,063	99.6	22.1
SW20-7	1	AC	2016	Saskatchewan	<i>T. durum</i>	35	6,568	50.9	7.2	76.2	13,116	99.4	22.5
SW2-1	1	AC	2016	Saskatchewan	<i>T. durum</i>	35.11	6,789	50.9	6.9	74.3	13,116	99.7	21.3
SW21-1	1	AC	2016	Saskatchewan	<i>T. durum</i>	34.62	6,282	50.9	6.8	74.3	12,965	99.4	21.6
SW21-7	1	AC	2016	Saskatchewan	<i>T. durum</i>	34.97	6,741	50.9	7	74.7	13,126	99.4	22.5
SW21-8	1	AC	2016	Saskatchewan	<i>T. durum</i>	34.96	6,631	50.9	6.9	75	13,073	99.4	22.2
SW7-5	1	AC	2016	Saskatchewan	<i>T. durum</i>	35.65	6,701	50.8	6.9	73.7	13,490	99.6	24.6
86-124	2	A	1986	Manitoba	<i>T. aestivum</i>	34.9	6,832	50.9	6.9	60.7	13,209	99.2	23
AB88-2	2	A	2010	Alberta	<i>T. aestivum</i>	34.83	6,465	50.9	6.9	75.3	13,126	99.5	22.5
L2-1	2	A	2016	Alberta	<i>T. durum</i>	34.96	6,923	50.9	7.1	72.9	13,130	99.4	22.5
SW1-2	2	A	2016	Saskatchewan	<i>T. durum</i>	35.08	6,599	50.9	7.1	71.2	13,365	99.4	24
SW15-1	2	A	2016	Saskatchewan	<i>T. durum</i>	34.83	6,272	50.9	6.6	78.8	13,201	99.7	22.9
T132-2	2	A	2017	Tunisia	<i>T. durum</i>	34.41	6,472	50.9	6.8	57.1	12,935	99.4	21.3
331-2	3	C	1985	Manitoba	<i>Triticum</i> sp.	34.44	6,828	51	6.9	55.6	12,909	99.5	21.2
D308	3	C	1985	Manitoba	<i>T. aestivum</i>	34.33	6,809	51	6.8	58.5	12,826	99.1	N/A <sup>4</sup>
D308*	3	C	1985	Manitoba	<i>T. aestivum</i>	39.6	70	50.8	18.6	3,667	12,501	99.6	18.7
I-72-1	3	C	2001	Syria	<i>Triticum</i> sp.	34.35	6,734	51	6.8	58.6	13,011	99.5	21.8
I-72-7	3	C	2001	Syria	<i>Triticum</i> sp.	34.35	6,696	51	7	58.9	12,901	99.4	21.2
SC29-1	3	C	1999	Saskatchewan	<i>T. durum</i>	34.19	6,464	51	6.8	59	12,951	99.5	21.5
SW21-5	3	C	2016	Saskatchewan	<i>T. durum</i>	34.66	6,619	51	7.2	63.5	13,029	99.5	22
90-2	4	absent	1990	Canada	<i>T. aestivum</i>	35.22	3,818	50.8	7.2	225.9	12,909	99.5	21.3
G9-4	4	absent	2016	Alberta	wild grass	36.97	8,035	50.7	9.8	78.2	13,148	98.7	23
T126-1	4	absent	2017	Tunisia	<i>T. durum</i>	34.15	6,373	51	6.5	62.4	12,837	99.6	20.6
92-171R5	5	B	1992	Saskatchewan	<i>T. aestivum</i>	36.81	14,647	51.1	10.2	45.1	13,393	99.3	24.1
Alg3-24	5	B	1993	Algeria	<i>T. durum</i>	34.3	6,098	50.9	6.6	73.6	12,900	99.6	21.1
Alg4x-1	5	B	1993	Algeria	<i>T. durum</i>	35.71	8,072	50.9	7.5	71	13,193	99.4	22.9
I-17-2	5	B	2001	Azerbaijan	<i>T. durum</i>	34.25	6,555	51	7	62.5	12,820	99.6	20.7
I-34-5	5	B	2001	Azerbaijan	<i>Triticum</i> sp.	34.29	6,315	51	6.8	61.9	12,841	99.6	20.8
I-35-56	5	B	2001	Azerbaijan	<i>T. durum</i>	34.24	6,516	51	6.9	62.6	12,918	99.5	21.3
I-36-1	5	B	2001	Azerbaijan	<i>T. durum</i>	34.36	6,467	50.9	6.7	62.4	12,881	99.5	21
AlgH1	6	BC	1993	Algeria	<i>T. durum</i>	34.74	6,902	51	6.9	61.7	13,159	99.3	22.7
AZ35-5	7	AB	2001	Azerbaijan	<i>T. durum</i>	35.3	7,165	50.9	7.1	72.1	13,239	99.5	23.2
T176-2	7	AB	2017	Tunisia	<i>T. aestivum</i>	34.7	6,896	50.9	7.3	57.1	13,141	99.5	22.6
T181-1	7	AB	2017	Tunisia	<i>T. durum</i>	34.78	6,718	51.2	6.5	57.5	13,583	99.4	25.1
I-34-1	8	ABC	2001	Azerbaijan	<i>T. durum</i>	34.38	6,405	50.9	6.6	64.5	13,036	99.5	22
I-35-18	8	ABC	2001	Azerbaijan	<i>T. durum</i>	34.85	6,698	50.9	7	65.3	13,071	99.4	22.9
I-73-1	8	ABC	2001	Syria	<i>T. aestivum</i>	34.62	6,619	50.9	6.8	63.4	12,941	99.5	N/A <sup>4</sup>
I-73-1*	8	ABC	2001	Syria	<i>T. aestivum</i>	39.9	39	50.8	18.3	3,647	12,744	99.6	20
T128-1	atypical	B	2017	Tunisia	<i>T. durum</i>	34.12	6,095	51	6.4	59.1	13,002	99.6	21.7

<sup>1</sup>NE= Necrotrophic effector; <sup>2</sup>Alberta, Saskatchewan, and Manitoba are provinces within Canada; <sup>3</sup>Long-read assemblies; <sup>4</sup>not included in pangenome

# Pangenome of Ptr



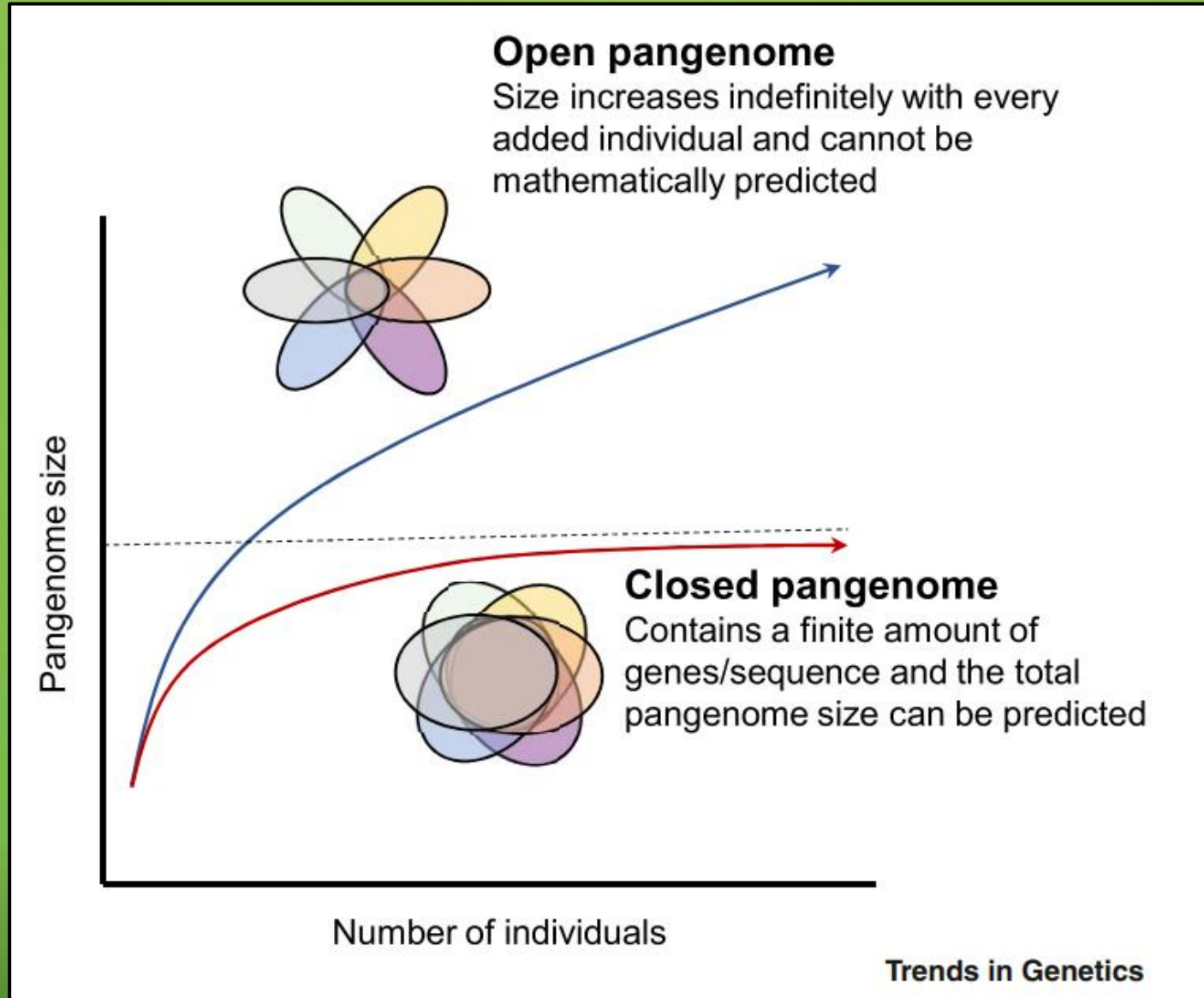
- 43% of genes in core set (all isolates)
  - 69% had functional info (Pfam)
  - CAZymes were highly conserved
- 57% of genes in accessory set (some isolates)
  - 28% had functional info (Pfam)
  - Effectors primarily accessory
- 25% of genes were singletons (subset of accessory in only one isolate)
  - Primarily from race 4 (non-pathogenic) and divergent race 5 (*ToxB*)
- Large accessory and singleton count
  - *Candida albicans*<sup>1</sup>: 91% core
  - *Zymoseptoria tritici*<sup>2</sup> (another wheat pathogen): 60% core
  - Previous Ptr pangenome<sup>3</sup> (11 isolates): 69% core
- Large accessory genome ~ diverse functions ~ higher adaptability

<sup>1</sup>*Microb. Genom.* **5**, 1-23 (2019)

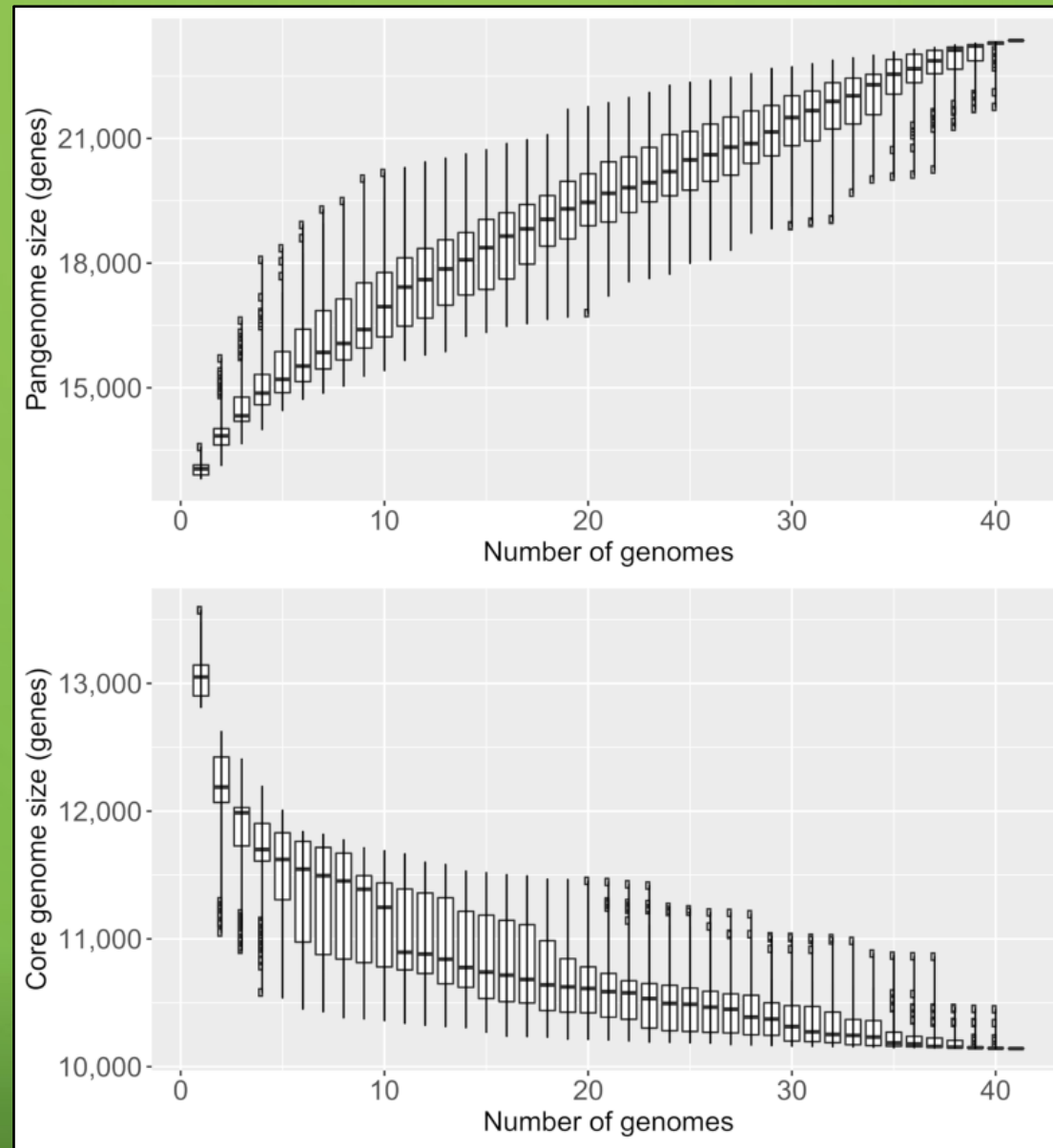
<sup>2</sup>*BMC Biology* **18**, 1-18 (2020)

<sup>3</sup>*BMC Genomics* **19**, 279 (2018)

# Pangenome of Ptr

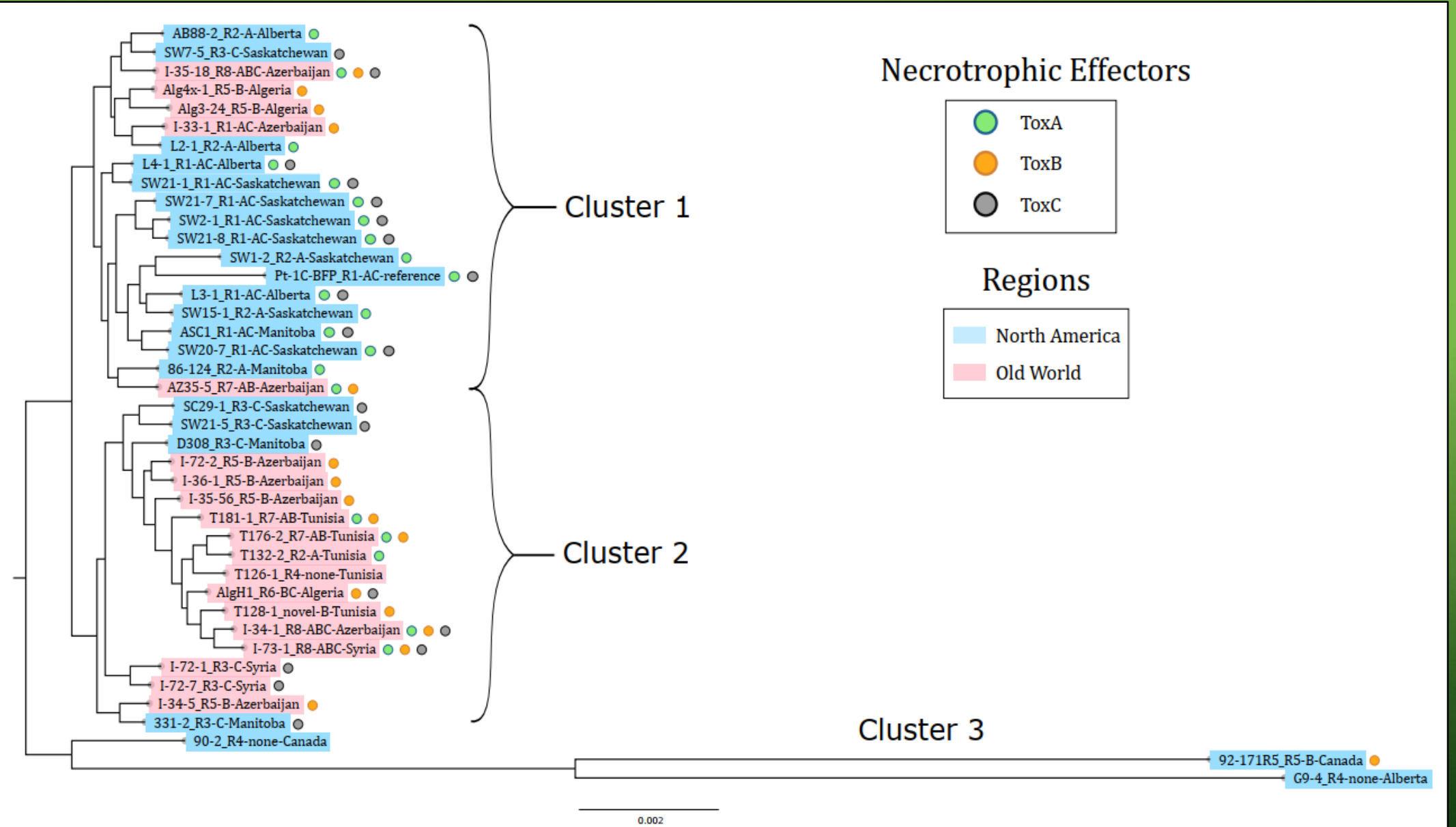


# Pangenome of Ptr

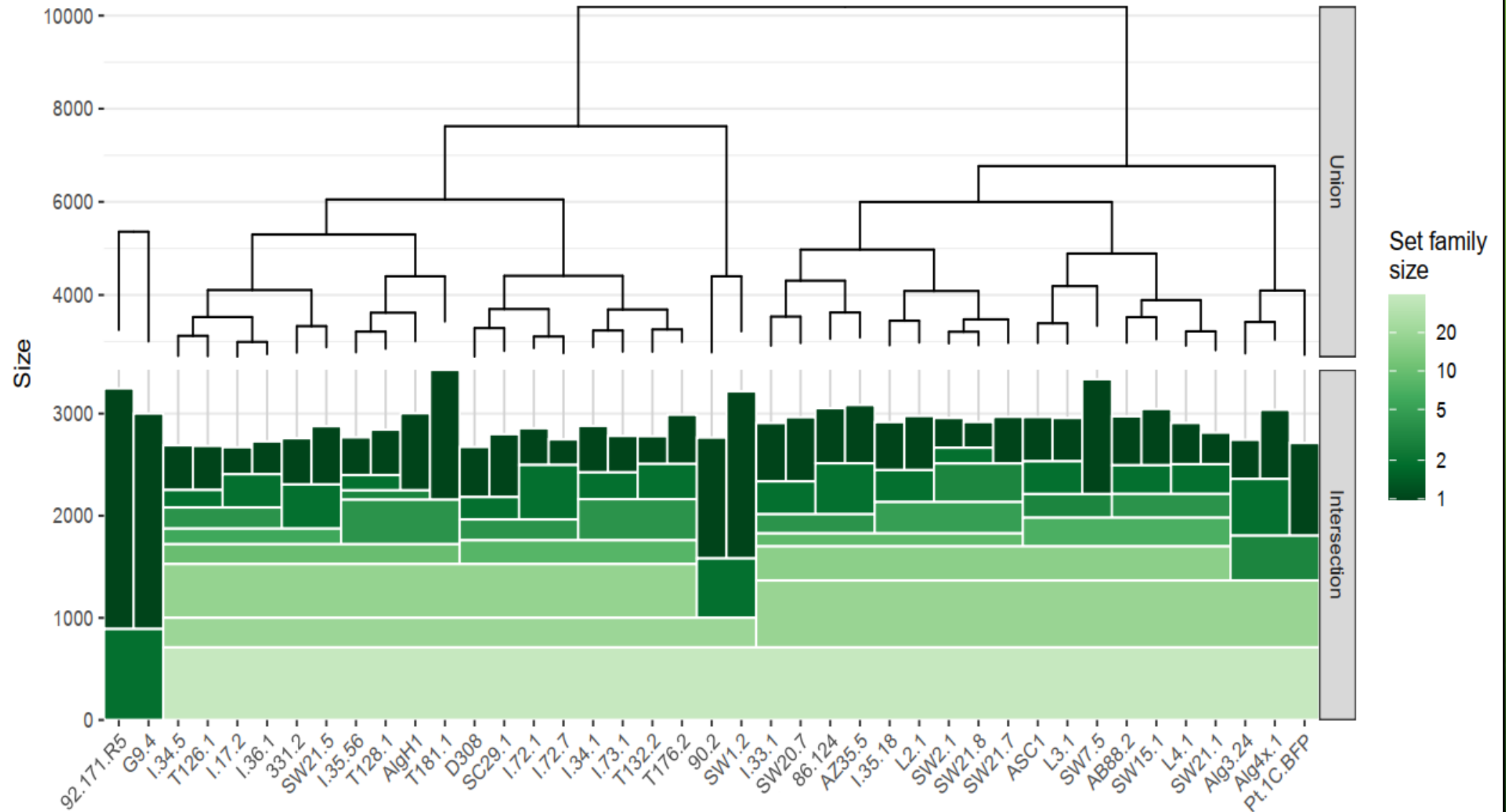




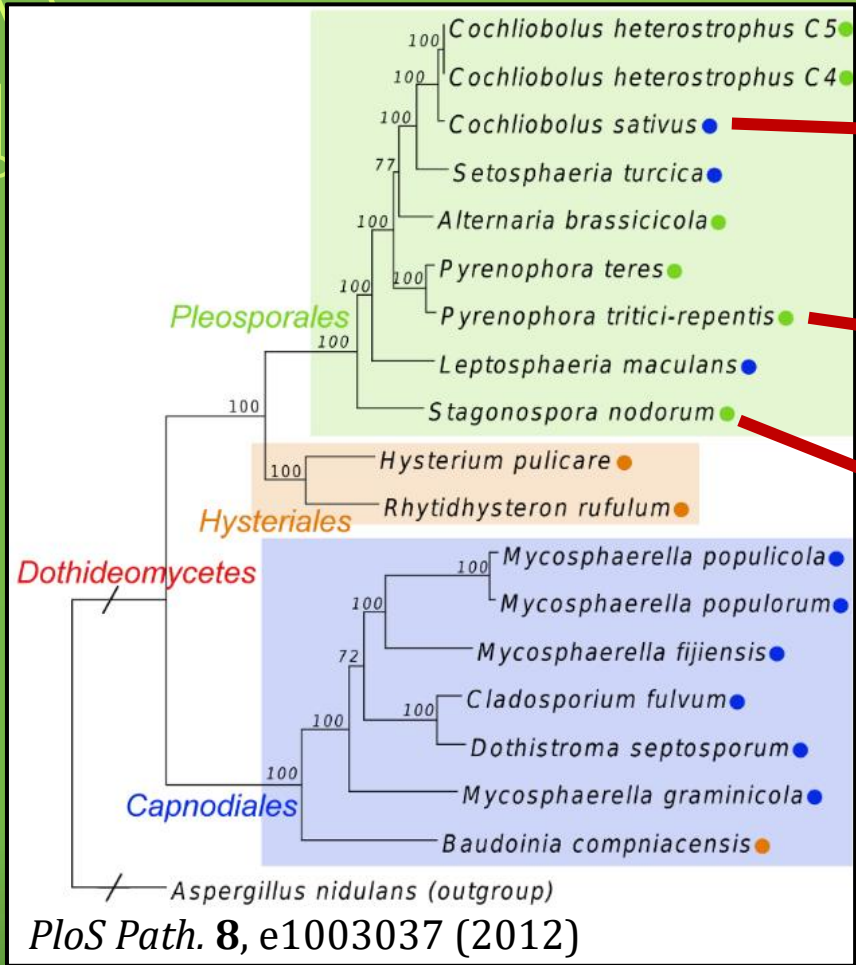
# Core protein phylogeny



# Accessory protein sets

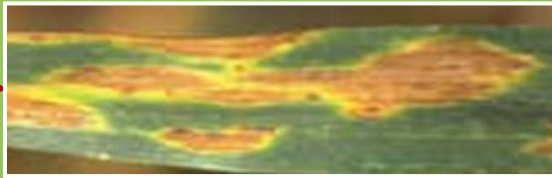


*ToxA* is present in other species



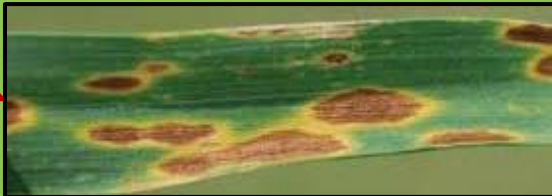
# Spot blotch

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



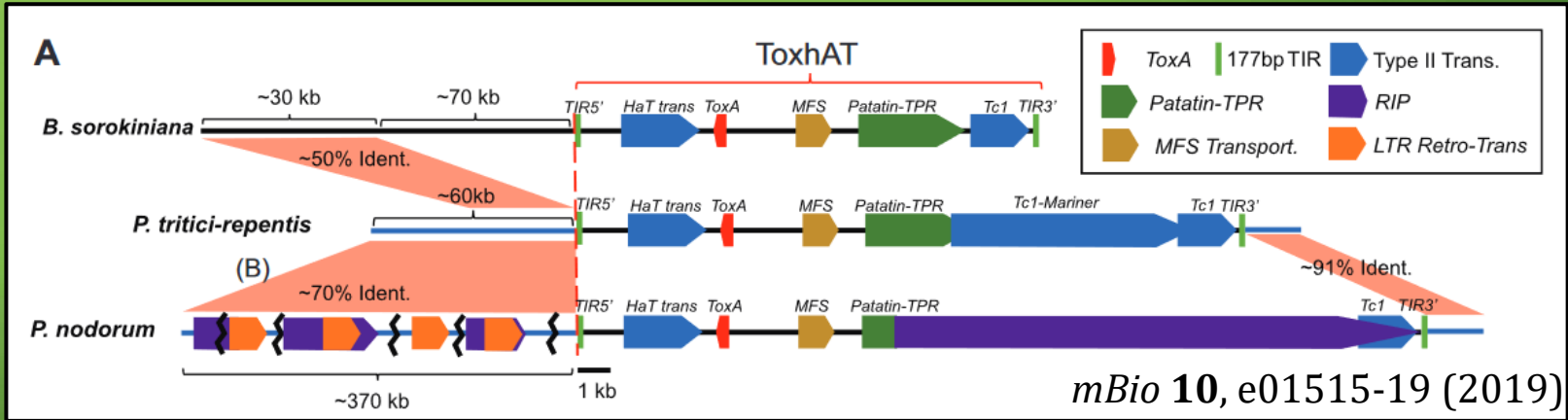
# Tan spot

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



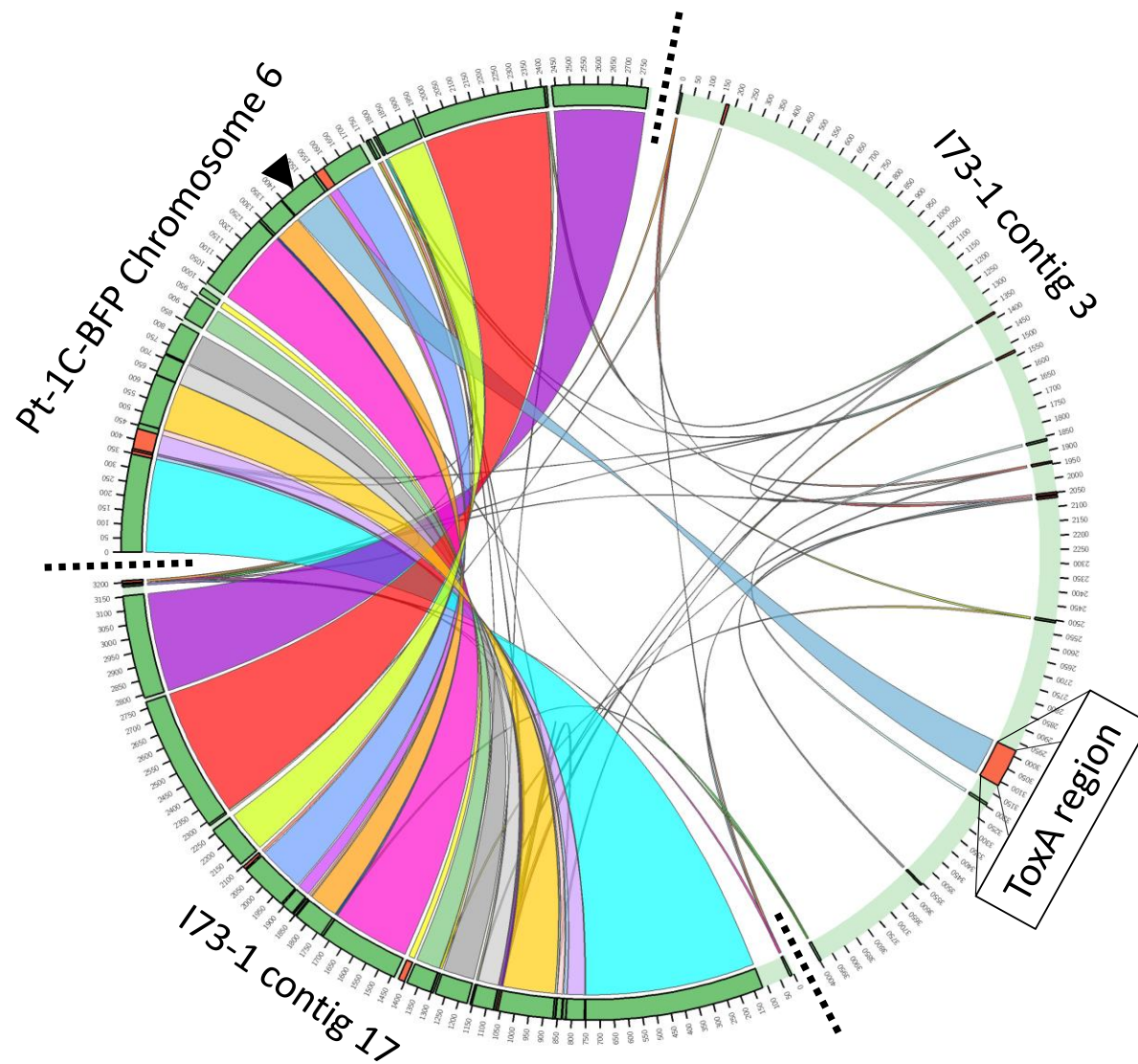
# Septoria blotch

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



*mBio* **10**, e01515-19 (2019)

# Intraspecific translocation of *ToxA*



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

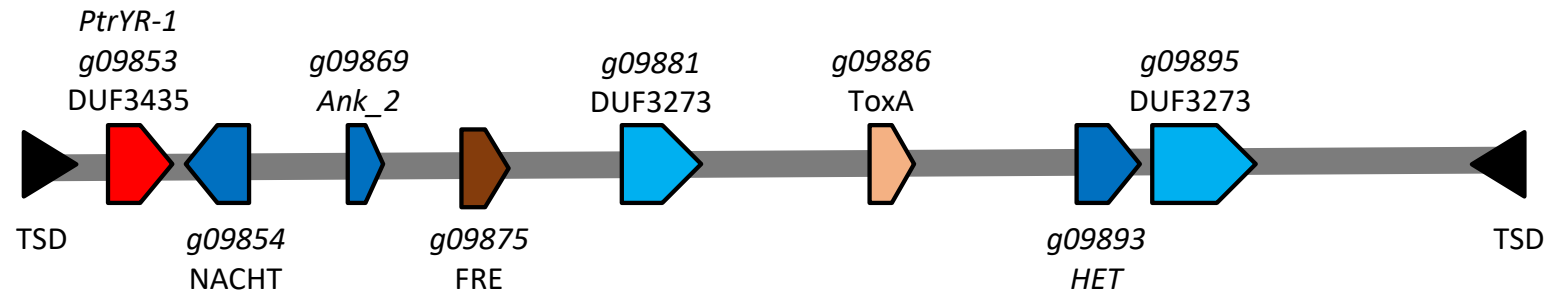
<sup>1</sup>Mol. Plant Pathol. **10**, 201-212 (2009)

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



# *ToxA* transposon is 'Starship' class

**I-73-1 contig 3**



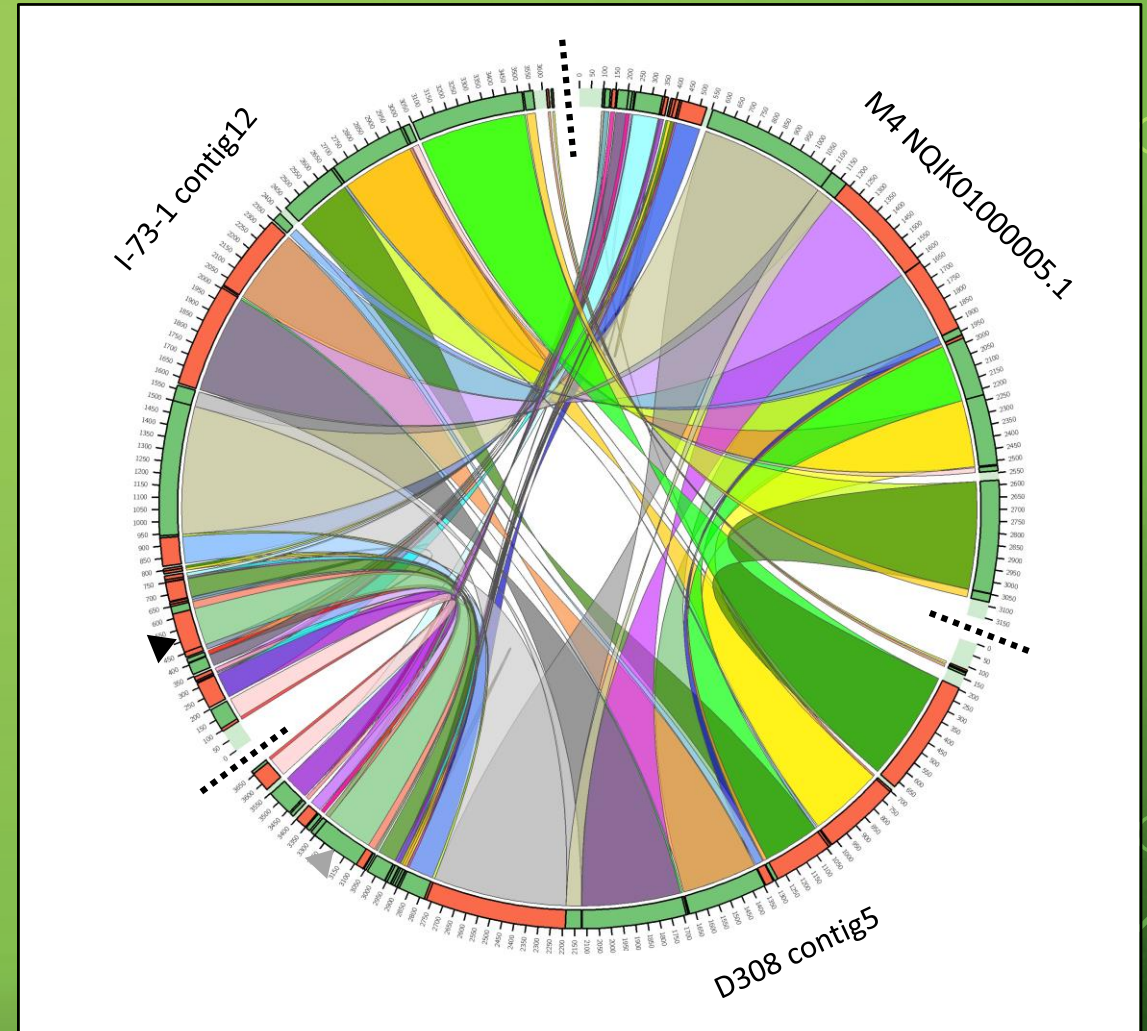
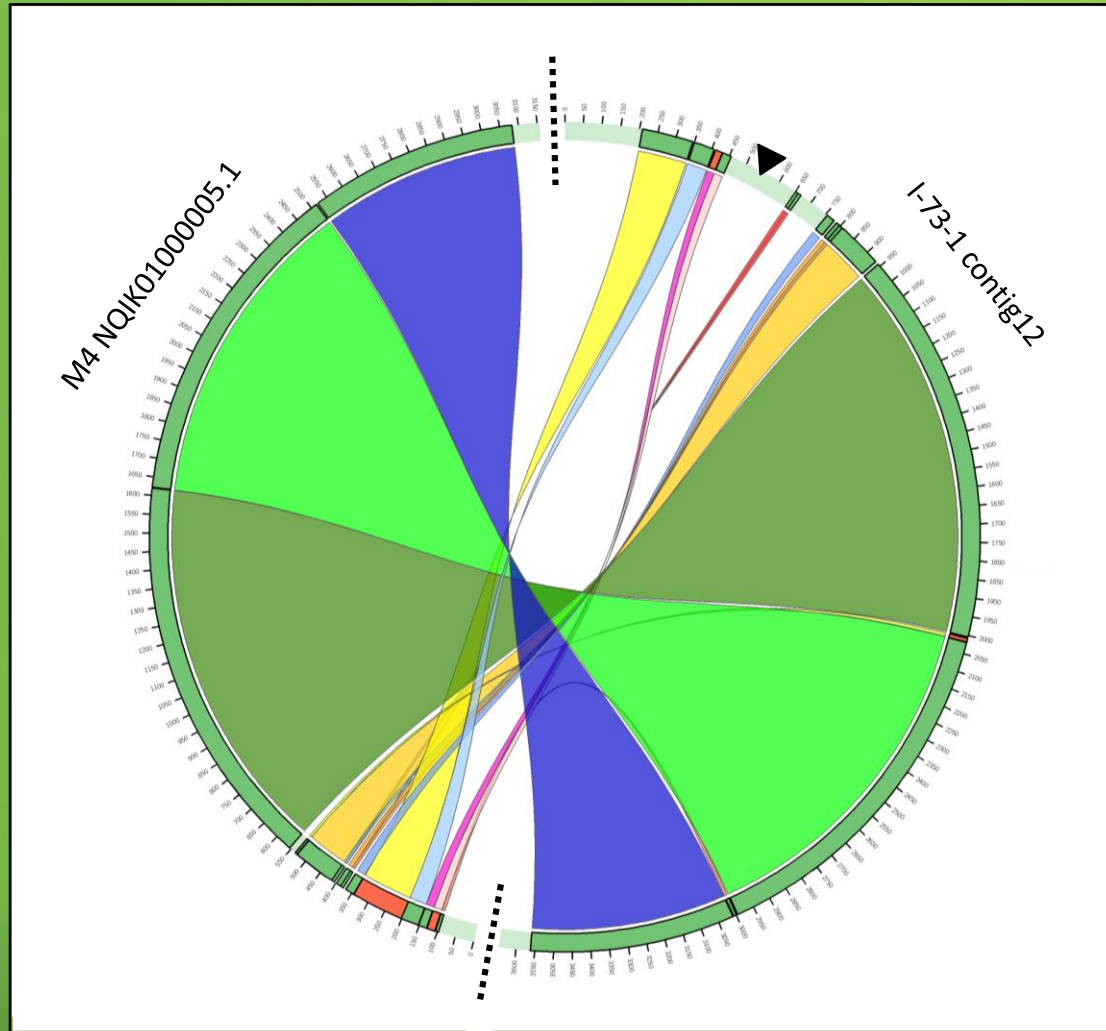
5' TTCACCTTTTACTAGAA---143kb---GAAATTTTTCACCAACCAA 3'

**Pt-1C-BFP chr1**

5' ---TATAAGTCTTTTGGTTGTAAAAGGTGAATGCGAGCAGGCA--- 3'

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

# Multi-copy *ToxB* on a putative transposon







# Summary of results and conclusions

- New high quality short-read assemblies representing all races of Ptr
- First long-read assemblies of race 3 (*ToxC*) and complex race 8 (*ToxA*, *B*, *C*)
- The Ptr genome is:
  - open and highly adaptive
  - plastic with chromosomal rearrangements between races (not presented)
  - ‘one-compartment’ but still maybe ‘two-speed’ (not presented)
- Phylogenetic clustering based on effector production and region
- *ToxA* is nested within multiple transposons: ToxhAT and new *Starship* class ‘Horizon’
- *ToxB* may also have transposon activity and mobility

# Preprint is now available

Available on ResearchGate and bioRxiv

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

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 D Wade Abbott,  Stephen Strelkov,  Fouad Daayf,  Reem Aboukhaddour

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Let us know what you think on twitter!

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@ropolo @sweetmicrobe @ReemWheat



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