Unpacking genomic mechanisms underpinning rapid adaptation of barley net blotch disease

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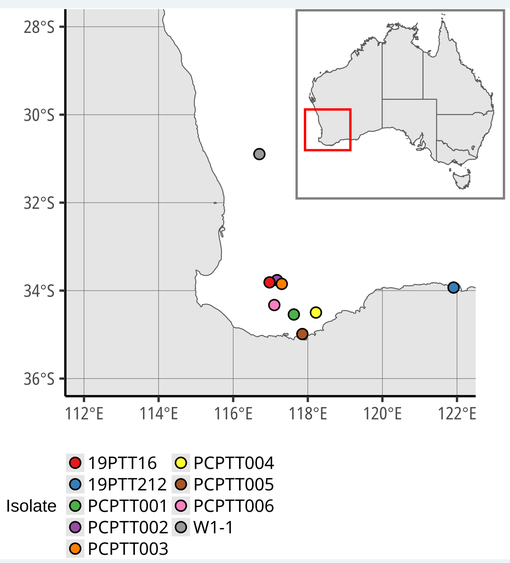
AAGI Analytics for the Australian Grains Industry

Department of Primary Industries and Regional Development, Government of Western Australia

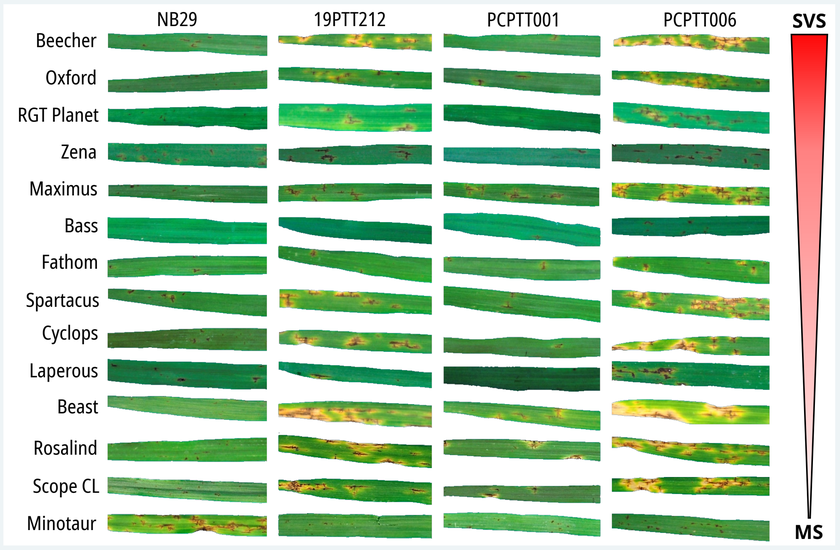


Introduction and background

- Net form net blotch disease of barley caused by fungal necrotroph ***Pyrenophora teres f. teres***
- Historical ***Beecher-virulent*** isolates were endemic[1], however 2016 epidemics saw the emergence of the 2019-characterised ***Oxford-virulent*** pathotype, and more recently ***RGT Planet-virulent*** pathotype in the southern growing regions of Western Australia
- **Eight** fungal isolates were sequenced from this growing region:
  - From 2019: **19PTT16** and 19PTT212
  - From 2023: **PCPTT001** - **PCPTT006**

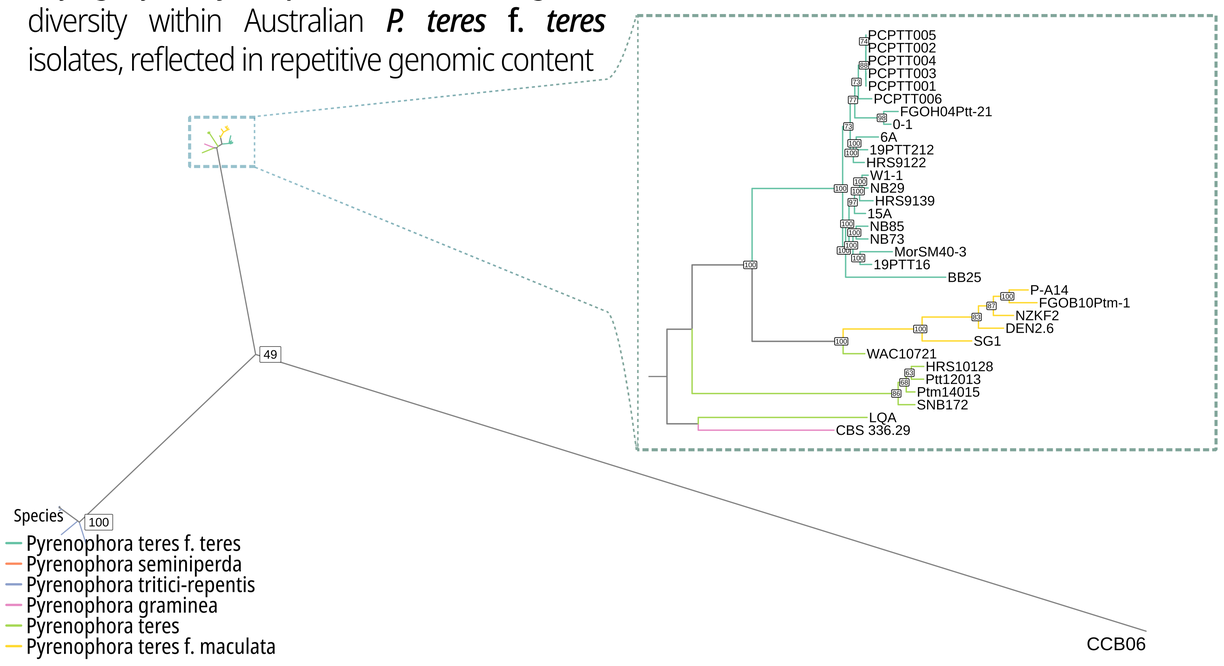


- Recent isolates found were found to be aggressively virulent on a barley cultivar infection assay, especially 2023 isolate **PCPTT006**
- Phenotyping confirmation of virulence raised the following question:
- What is the genetic basis of increased virulence?

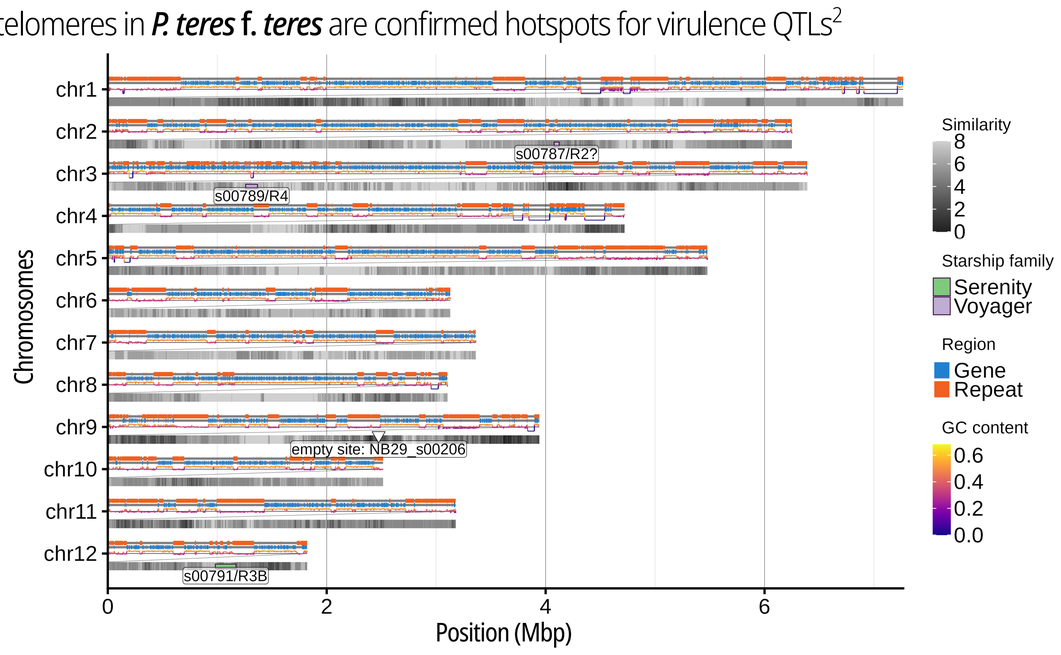


Evidence for a shift in genome organisation

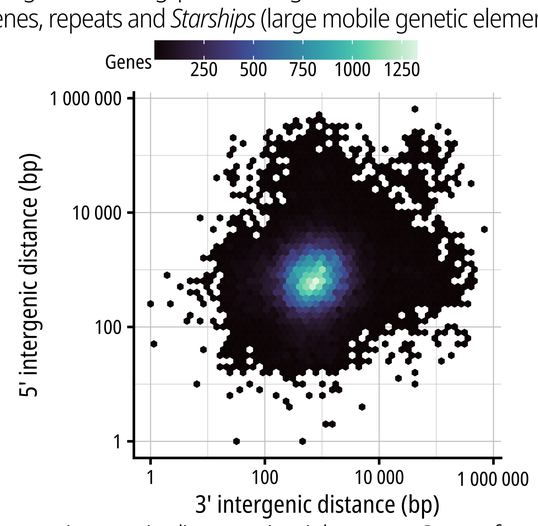
- Phylogeny of ***Pyrenophora*** reveals rich global diversity within Australian ***P. teres* f. *teres*** isolates, reflected in repetitive genomic content



- Direct pairwise comparisons of 2019 and 2023 isolates against 1985 Western Australian isolate **W1-1** revealed extensive divergence within repeat islands, frequently localised to subtelomeres
- Subtelomeres in ***P. teres* f. *teres*** are confirmed hotspots for virulence QTLs[2]



Ideogram showing pairwise alignment of 2019 and 2023 *P. teres* f. *teres* isolates against 1985 isolate W1-1, showing genes, repeats and *Starships* (large mobile genetic elements)



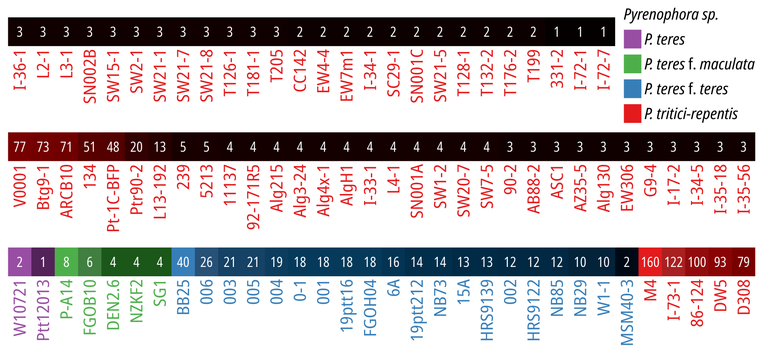
Average intergenic distances in eight recent *P. teres* f. *teres* isolates

- An analysis of intergenic distances supports a shift in the regulation of transposon proliferation via **Repeat Induced Point mutations (RIP)**[3]
- This finding mirrors similar shifts identified in ***Zymoseptoria tritici*** and sister species ***P. tritici-repentis***, along with implicated shifts in fungal pathogenicity and lifestyle[4,5]

28.1% RIP-affected regions → **25.6%** in 2023 isolates

Three emergent potentiators of virulence

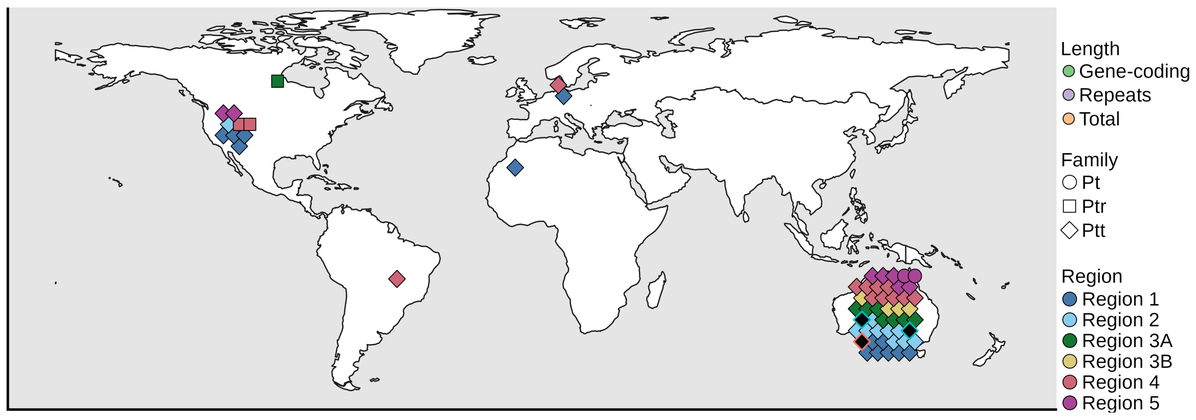
1 - *hAT-Tnp dimer* proliferation



Counts of *hAT-Tnp dimer* transposons across *Pyrenophora*

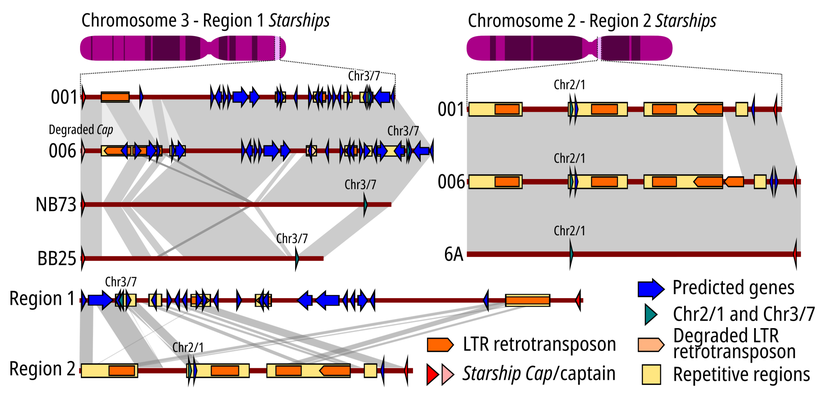
- *hAT-Tnp dimer* transposons are ubiquitous to *Pyrenophora*
- Identification and BLAST hits reveal enrichment in virulent, effector-producing isolates and recent *P. teres f. teres*[6]
- Previously identified to be associated with chromosomal rearrangements[7]

2 - *Starships* carry putative virulence



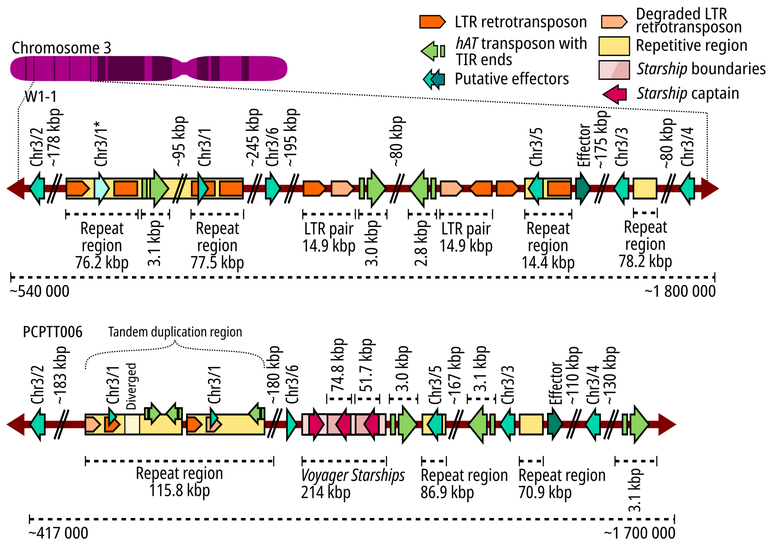
Distribution of six manually curated *Starships*, based on flanking genomic similarity

- A screen of ~2600 *Dothideomycete* genomes revealed that *Starships* are globally distributed within *Pyrenophora*
- Manual curation of flanking regions confirmed 6 unique elements, interspecific and virulence-encoding (Reg. 4)
- Reg. 1 and 2 *Starships* encode a similar **candidate effector**, only **2023 isolates** carry both unique regions



Annotations of Region 1 and 2 *Starships* encoding a similar candidate effector

3 - Candidate effectors in proximity to *hAT* and *LTR* transposons



- The virulence associated sub-telomere of chromosome 3 (with known QTLs) was screened using fine-mapped **RNA-seq** data
- Seven **candidate effectors** were identified, expressed *in planta*
- Chr3/1 was found to within a duplicated segment, complete with ***hAT-Tnp dimer*** and ***LTR*** transposons at the segment boundaries
- Eight other expressed candidates were identified throughout the genome

References

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