

# The next-generation of blackleg control

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# Symptoms of *L. maculans* and *L. biglobosa*

## *L. maculans*

## *L. biglobosa*

7 days



Penetration



Nutrient acquisition

14 days



Colonisation



Asexual sporulation

End of season



Degradation of tough stem tissue



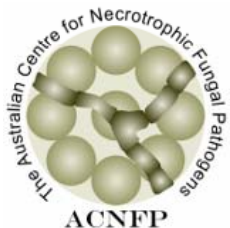
End summer

Sexual sporulation

# *L. maculans* genome

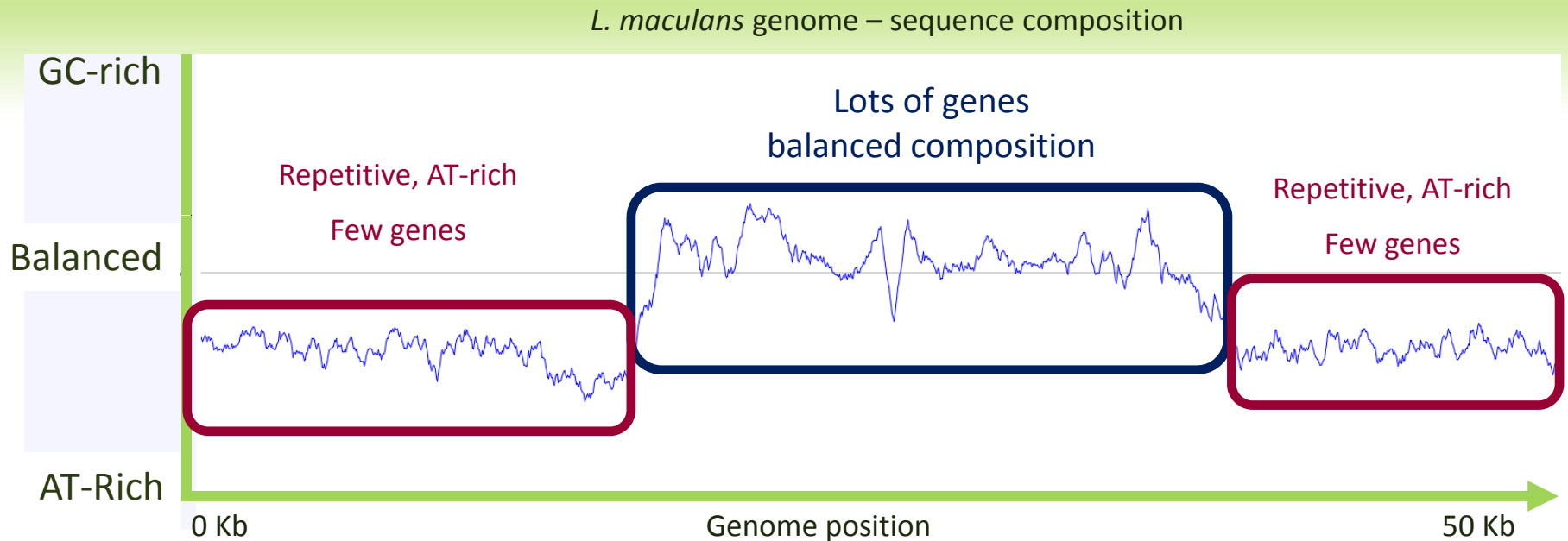


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- ⊙ High-quality genome, multi-national project, large investment
- ⊙ Published in Nature Communications Feb 2011
- ⊙ 12,500 genes; 45 Million base-pair genome
  - ⊙ Closely related to the wheat glume-blotch fungus (*S. nodorum*)
- ⊙ Lots of Repetitive DNA: 36% genome (9% of *S. nodorum*)
- ⊙ Degenerated transposable elements (“jumping genes”)

# The "Patchwork" genome of *L. maculans*

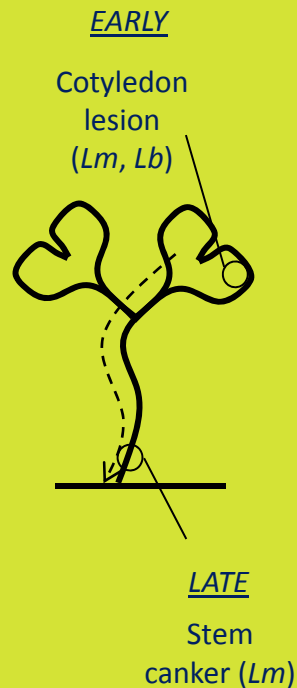


- ◎ Repeat-rich blocks
  - readily gained and lost
  - environment for new genetic variation
  - avirulence effector genes such as *AvrLm1*
- = Rapid adaptation to the plant genotype

# *L. biglobosa* has a compact genome

- ◎ Next-generation sequencing of *L. biglobosa* genome
- ◎ Fewer genes, compact genome
- ◎ Far fewer repeat sequences in *L. biglobosa*

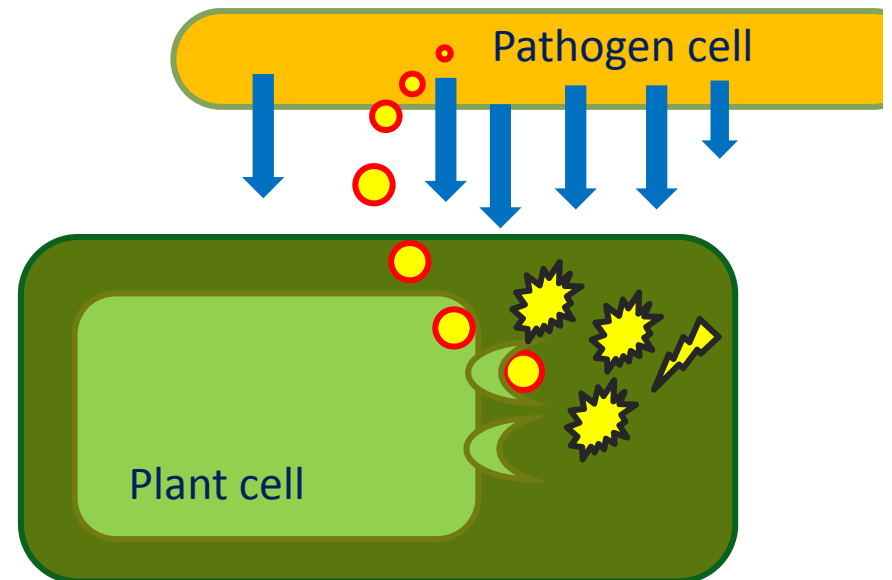
Canola Seedling



	<i>L. maculans</i>	<i>L. biglobosa</i>
Disease status	Important	Emerging?
Cotyledon infection	Slow	Fast
Stem canker	Frequent	<b>Rare</b>
Genome size	45 Mb	<b>30 Mb</b>
Repetitive DNA sequences	Many	<b>Few</b>
Predicted genes	12,469	9,610

# Disease genes in *L. maculans* & *L. biglobosa*

- ⊙ **A major class of disease genes are those encoding “Effectors”**
- ⊙ Effectors
  - ⊙ have major roles in disease establishment and progression
  - ⊙ often interfere with plant defence
- ⊙ are small secreted proteins that interact with the host
- ⊙ Can be predicted directly from a genome sequence







# The next-generation of blackleg control

- ◎ A rapidly adapting pathogen means we must find more targets for control
- ◎ Next-generation genomics enables a new approach to Blackleg research
- ◎ Two ways of identifying new disease genes
  - ◎ Predict effectors based on genome sequence
  - ◎ Use similarity to known disease-genes in other fungi



## *L. maculans* has more effector genes

- ⊙ Predicted effectors based on genome sequence
- ⊙ Most effectors are specific to that species

	<i>L. maculans</i>	<i>L. biglobosa</i>
Effectors	651	362
Unique Effectors	292	93



## *L. maculans* has more effector genes



Most effectors are specific to that species

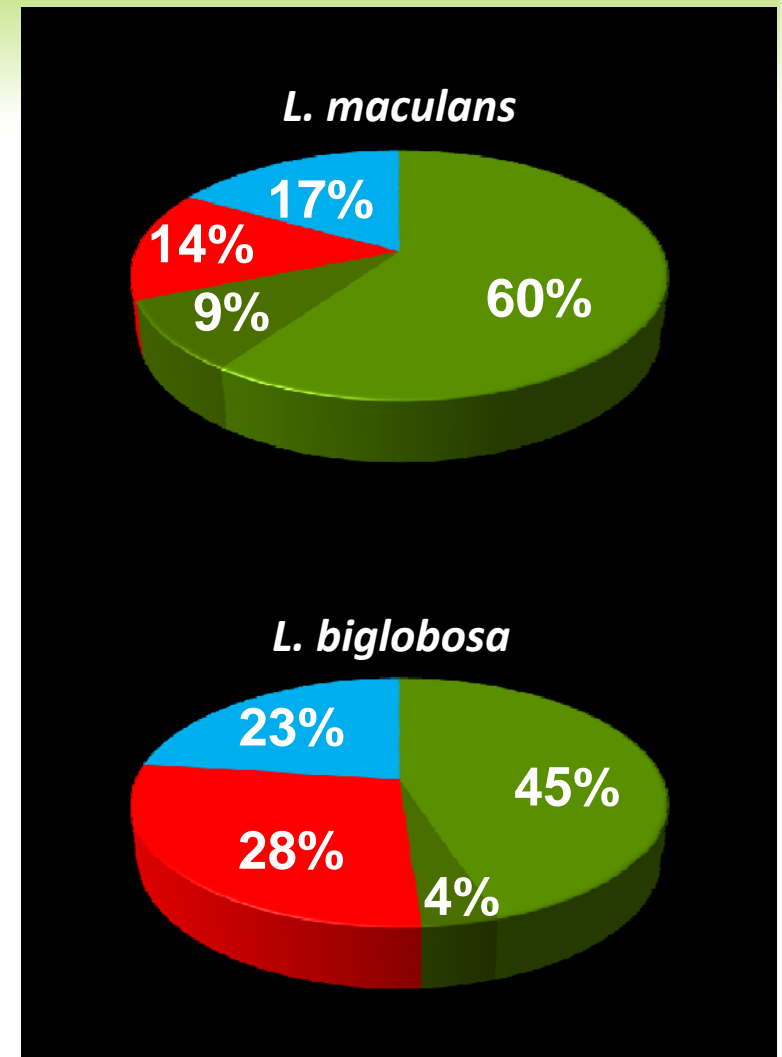



~20% have a pair in the other *Leptosphaeria*.



~20% belong to extended families in both species

- ⊙ ***Selected candidates will be tested on canola***
- ⊙ ***Genes with a contribution to disease will be identified***





# Using the Pathogen-Host Interactions database to find disease genes

- ⊙ The Pathogen-Host Interactions database (PHI-BASE)
- ⊙ Experimentally verified disease genes only
- ⊙ Expert curation, experimental evidence
- ⊙ I compared the genes of *L. maculans*, *L. biglobosa*, *S. sclerotiorum* with all PHI-BASE genes
- ⊙ Examples where there were **relatives in *Leptosphaeria* spp.** but **not in *Sclerotinia*.**

# A small subset of PHI-BASE genes are specific to *Leptosphaeria*

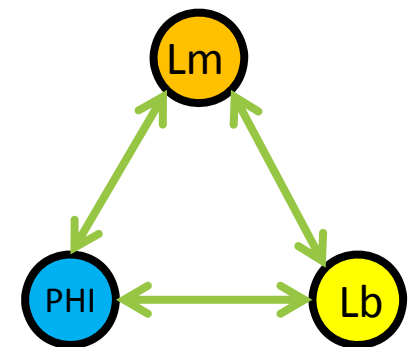
Whole genome

	PHI-BASE	<i>L. maculans</i>	<i>L. biglobosa</i>	<i>S. sclerotiorum</i>
Total Genes	923	12,469	9,610	14,503
Included in analysis	530	<b>233</b>	<b>227</b>	<b>261</b>

Subset of PHI-BASE matches

Type of match	<i>L. maculans</i> genes	<i>L. biglobosa</i> genes
Lmac & Lbig	21	19
Lmac only	9	-
Lbig only	-	7

Few candidate genes





## A small subset of PHI-BASE genes are specific to *Leptosphaeria*

- ◎ PHI-BASE-related disease genes identified in *Leptosphaeria*
- ◎ 3 genes in *L. biglobosa* only
  - ◎ Cell-wall penetration/degrading enzymes
- ◎ 4 genes in *L. maculans* only
  - ◎ Protein degradation, Toxin metabolism
- ◎ 18 genes in both *Leptosphaeria* spp.
  - ◎ Phytoalexin breakdown (anti-microbial, similar to Brassinin)  
Oxidative stress resistance, 5 matches to Rice blast genes

# Functional testing of potential control targets

**Select**

genes from genome sequence and PHI-BASE database

**Validate**

gene activation during disease (Next-Gen RNA Sequencing)

**Purify**

recombinant protein from the gene in the lab

**Apply**

protein to cotyledons or leaves record lesion development

**Mutate**

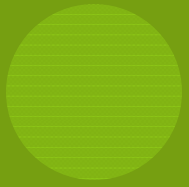
*Leptosphaeria* to “Silence” the genes

**Assess**

virulence of silenced mutants on canola



**Knowledge of protein function is critical to understand the underlying mechanisms of blackleg disease**



③ Any questions?