

How did *Leptosphaeria maculans* become such a successful pathogen?

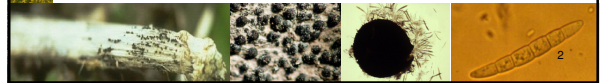


- Barbara Howlett
- School of Botany, the University of Melbourne



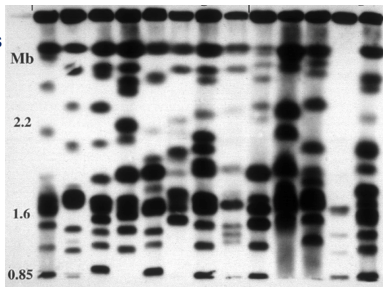
Blackleg fungus, *Leptosphaeria maculans*

- Sexual reproduction on stubble generates extremely large populations of wind-borne recombinant ascospores
- Prolific asexual crossing
- Large inoculum of genetically diverse individuals
- Populations readily adapt to selection pressure from extensive sowing of cultivars with same resistance genes
- Frequency of virulent isolates increases, leading to breakdown of resistance; eg. Eyre Peninsula in 2003



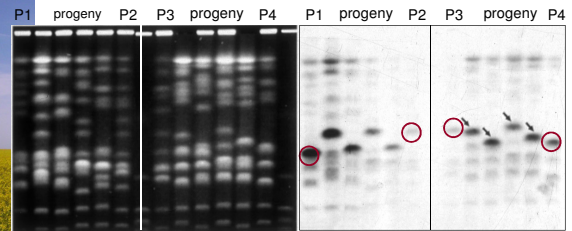
Genome structure: Isolates have different-sized chromosomes (chromosomal length polymorphisms)

18 chromosomes
Genome size ca. 40 Mb



Plummer & Howlett (1993) Current Genetics

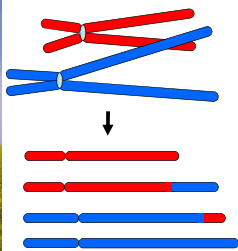
Progeny can have different sized homologous chromosomes to those of parents



Same size as parents Different size to parents

Plummer & Howlett (1995) Current Genetics

Inheritance of different sized homologous chromosomes

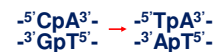


Hypothesis: Chromosomal length polymorphisms due to different amounts of **repetitive DNA** in homologs

Slippage (unequal pairing) during meiosis (during crossing) leads to generation of length polymorphisms

Mutation: Repeat Induced Point (RIP)

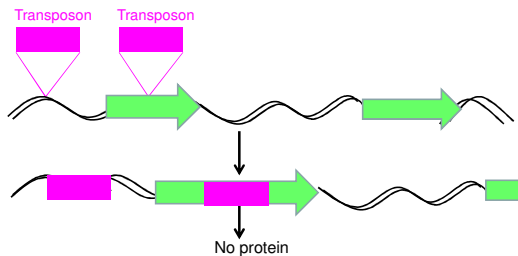
- *L. maculans* undergoes Repeat Induced Point (RIP) mutation
- RIP mutates **repetitive DNA** and **multicopy** genes in haploid nuclei after fertilization; thus only occurs during sexual crossing
- Changes from C:G to T:A base pairs. Often **generates stop codons**; thus genes are not expressed
- RIP mutation 'signature' can be deduced by bioinformatic analysis of genome sequences
- Demonstrated experimentally in
- Defence against transposable elements?



Idnurm & Howlett (2003) Fungal Genetics & Biol.

Transposable elements (transposons) may be source of repetitive DNA

segments of repetitive DNA that can mobilize and propagate within host genomes and inactivate genes

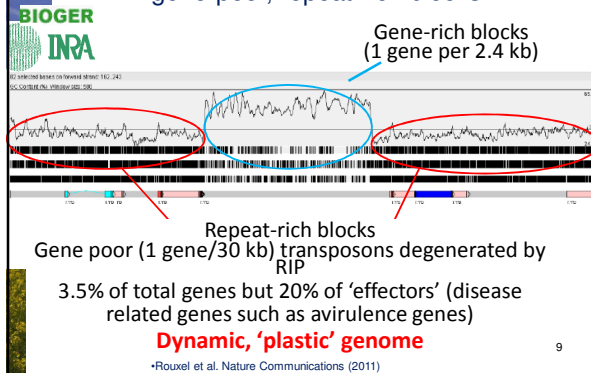


Leptosphaeria maculans genome



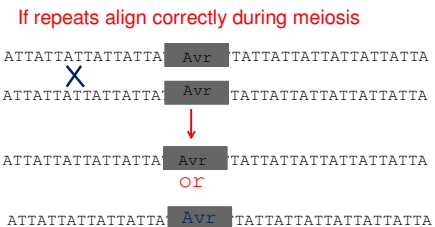
- Rouxel & Balesdent, INRA, France, Howlett, UniMelb, Oliver, Murdoch Uni, Genoscope; URGI, France; 2011
- 12,500 genes; 45 Mb -closely related *Stagonospora nodorum* has genome size of 37 Mb
- Repetitive DNA: 36% genome (9% *S. nodorum*); Degenerated & truncated transposable elements
- Repeat Induced Point (RIP) mutation
- Unique 'patchwork' genome structure

Unique 'Patchwork' genome: gene-rich & gene-poor, repeat-rich blocks

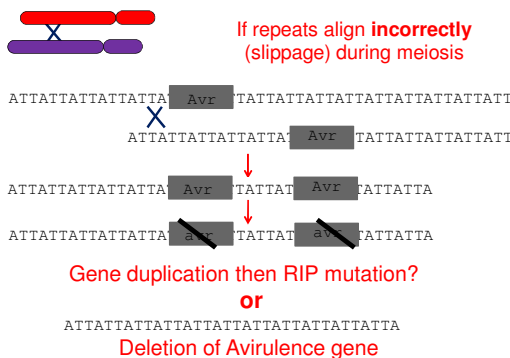


Location of avirulence genes in repetitive regions can lead to gene loss or mutation

Meiosis occurs during sexual reproduction
Sister chromatids align
Crossovers can occur (recombination)



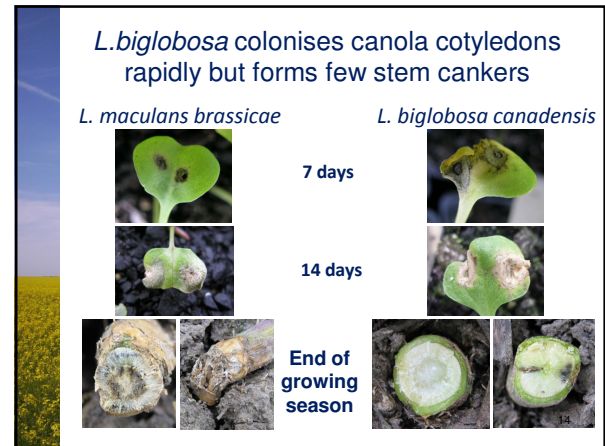
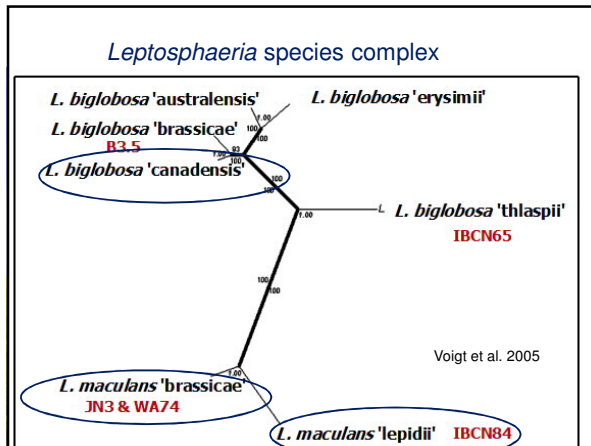
Location of avirulence genes in repetitive regions can lead to gene loss or mutation



Resistance breakdown and evolution of virulence

- Virulence evolves so rapidly in the blackleg fungus because avirulence genes are located in repetitive regions of genome where RIP mutation and gene loss occur readily
- Loss (deletion) of avirulence gene *AvrLm1* in genomes of populations of blackleg fungus is responsible for breakdown of Sylvestris resistance in 1993 on Eyre Peninsula
- RIP mutation can mutate single copy genes, such as *AvrLm6*, due to 'leakage' during mutation of repetitive DNA

(Van de Wouw et al. 2010 PLoS Pathogens)



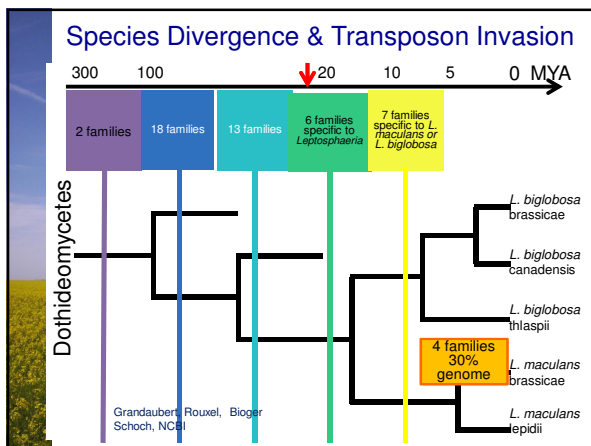
- ### Comparing *Leptosphaeria* genomes
- Collaboration: Rouxel, France; Howlett, Australia; Schoch, USA; Borhan, Canada
 - Sequenced genomes of five *Leptosphaeria* 'species'
 - Compared sequence 19 genes (not under selection pressure) in 51 related fungi
 - **Questions**
 - Are other *Leptosphaeria* 'species' transposon-rich?
 - How prevalent is RIP mutation?
 - What is number and species-specificity of effectors in members of 'species' complex?
 - When did *Leptosphaeria* 'species' diverge from each other?
 - When did transposable elements invade *L. maculans*?

Leptosphaeria genomes

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Lowe

	Lmb	Lml	Lbt	Lbb	Lbc *
Genome Mb	45	31.5	32.1	31.8	30.2
Transposable Elements %	36	2.7	4	3.2	2.9
Effectors	651	737	696	665	621

- Each fungus: 12,500 genes and RIP mutation
- Gene order in *L. maculans brassicae* (Lmb) same as in *L. maculans lepidii*, but transposable elements between genes in Lmb
- *L. maculans brassicae* (Lmb) has twice as many species-specific effectors as other species
- 47 families of transposable elements



- ### How did *Leptosphaeria maculans* become such a successful pathogen?
- **Biology**
 - Prolific sexual reproduction: many recombinants
 - Evolved from less aggressive fungi
 - **Genome Structure and Mutation**
 - 'Recent' massive invasion of transposons and subsequent Repeat Induced Point (RIP) mutation sculpted 'patchwork' genome where inactivated transposons house genes involved in disease (effectors)
 - Chromosome slippage during meiosis (during crossing) occurs. Thus loss /gain of effectors
 - RIP mutation still occurs during crossing on stubble and inactivates single copy effector avirulence genes
- Australian Government
GRDC Grains Research & Development Corporation