Phytophthora capsici

Loss of Heterozygosity (LOH): A Widespread Mechanism for Rapid Adaptation

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P. capsici phylogenetic context

Class

- Oomycete (eukaryotic)
- Looks like a fungus, acts like a fungus, but isn't a fungus

Genus

- Phytophthora = Plant Destroyer
- Explosive Epidemiology (asexual spores)

Species

- Sexually-recombined oospores can remain dormant for years
- Survives on living or dead tissues
- Remarkable adaptation to fungicides and new hosts
- Model: culture all life stages, sexually fecund, genome sequence

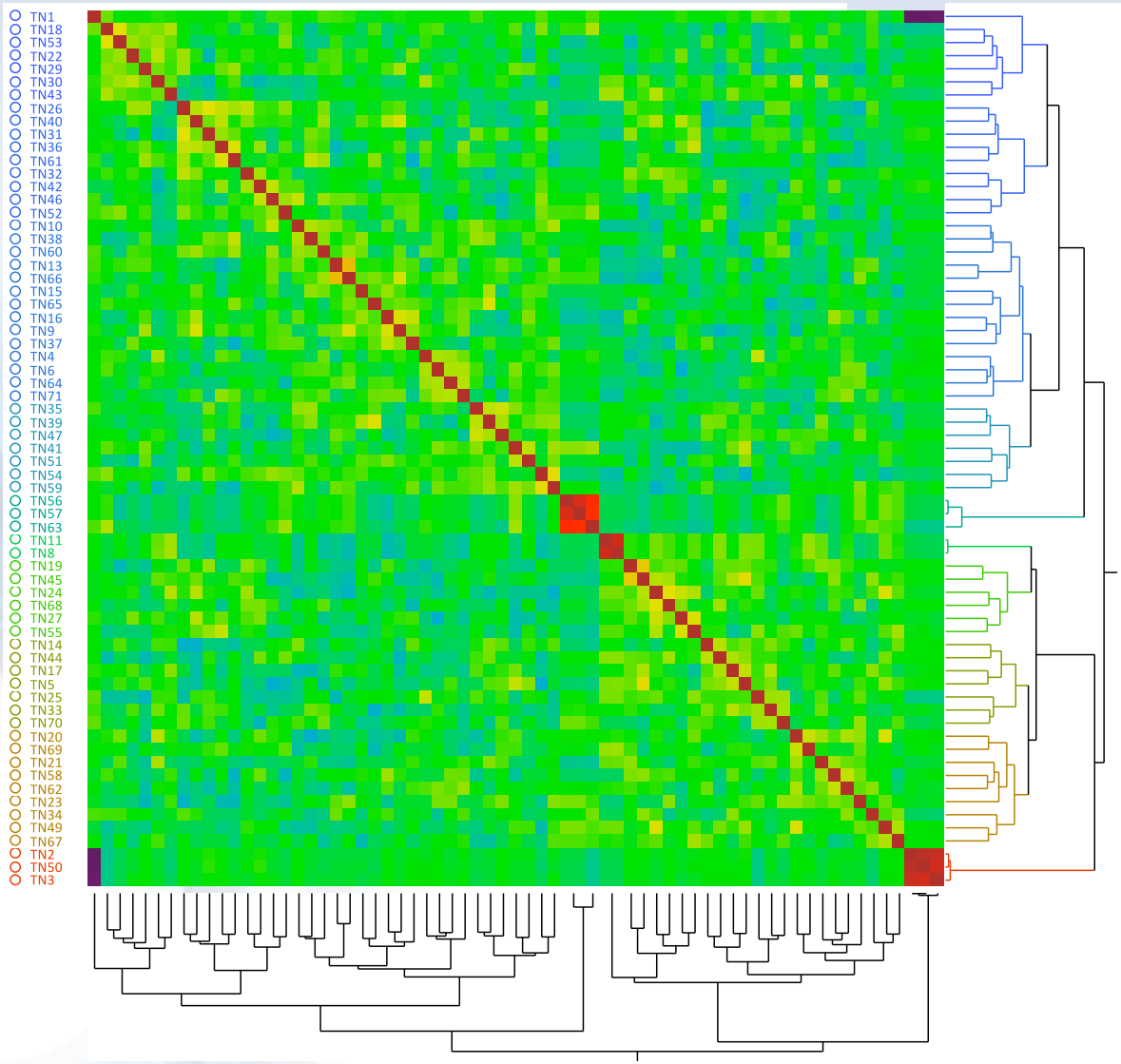
Restriction site Associated DNA (RAD-Seq) 69 Progeny and 2 Parents

	Parents and Progeny	Parents	Progeny
Total	158,460,781	9,588,155	148,872,626
Mean	2,231,842	4,794,078	2,157,574
Median	1,655,467	4,794,078	1,646,382
StDev	1,602,718	138,864	1,563,741
Min	594,851	4,695,886	594,851
Max	8,951,606	4,892,269	8,951,606

Markers

- Genotypes at 500,352 sites
- 20,568 mendelian SNPs mapped
  - 1 marker every 25 bp

Coding Silent	Coding Non-Silent	Intron	3'	5'	Non-Gene	Genes Hit
5872	3382	1993	1310	360	7651	5017



Genetic Similarity

(11K markers)

- ✓ 4 had low coverage or significant contamination.
- ✓ 2 were clones of the TN parent
- ✓ 5 were part of 2 different progeny clonal lineages.
- ✓ 60 true sexual progeny

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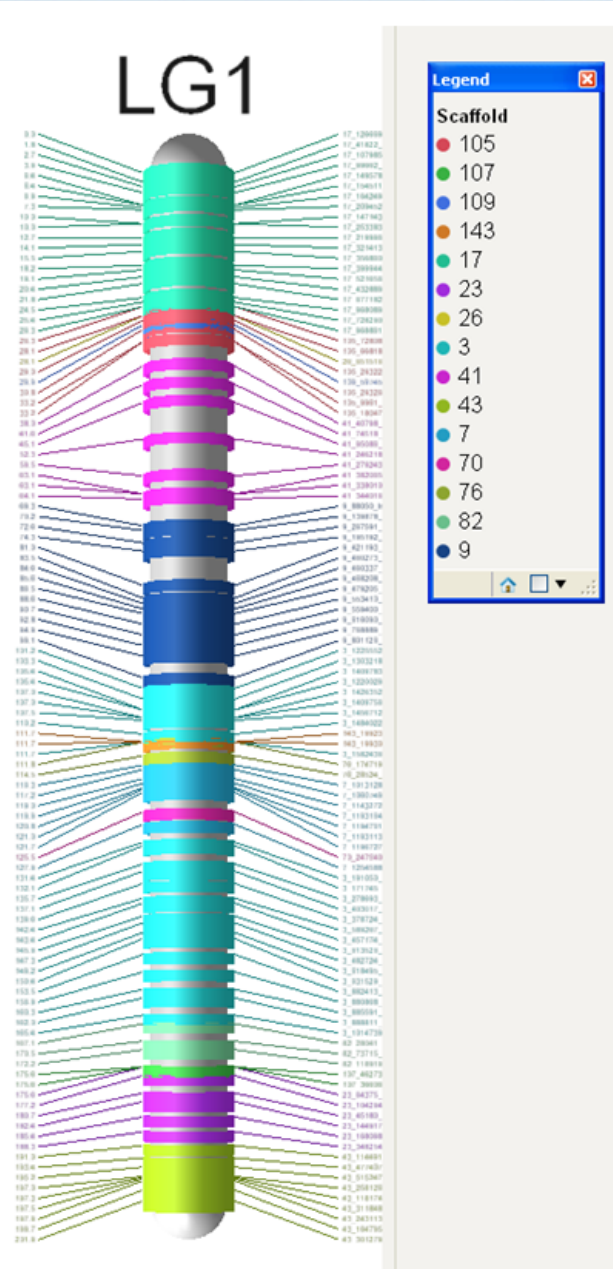
Genetic Linkage Analyses (JoinMap 4.1)
CP cross



Scaffold 'Blocks'

Scaffold Block	Sc23.1	Sc23.2
Lowest Marker	23_45180	23_653052
Highest Marker	23_357600	23_764546
Total Covered	312,420	111,494
Linkage Group	LG1	LG2

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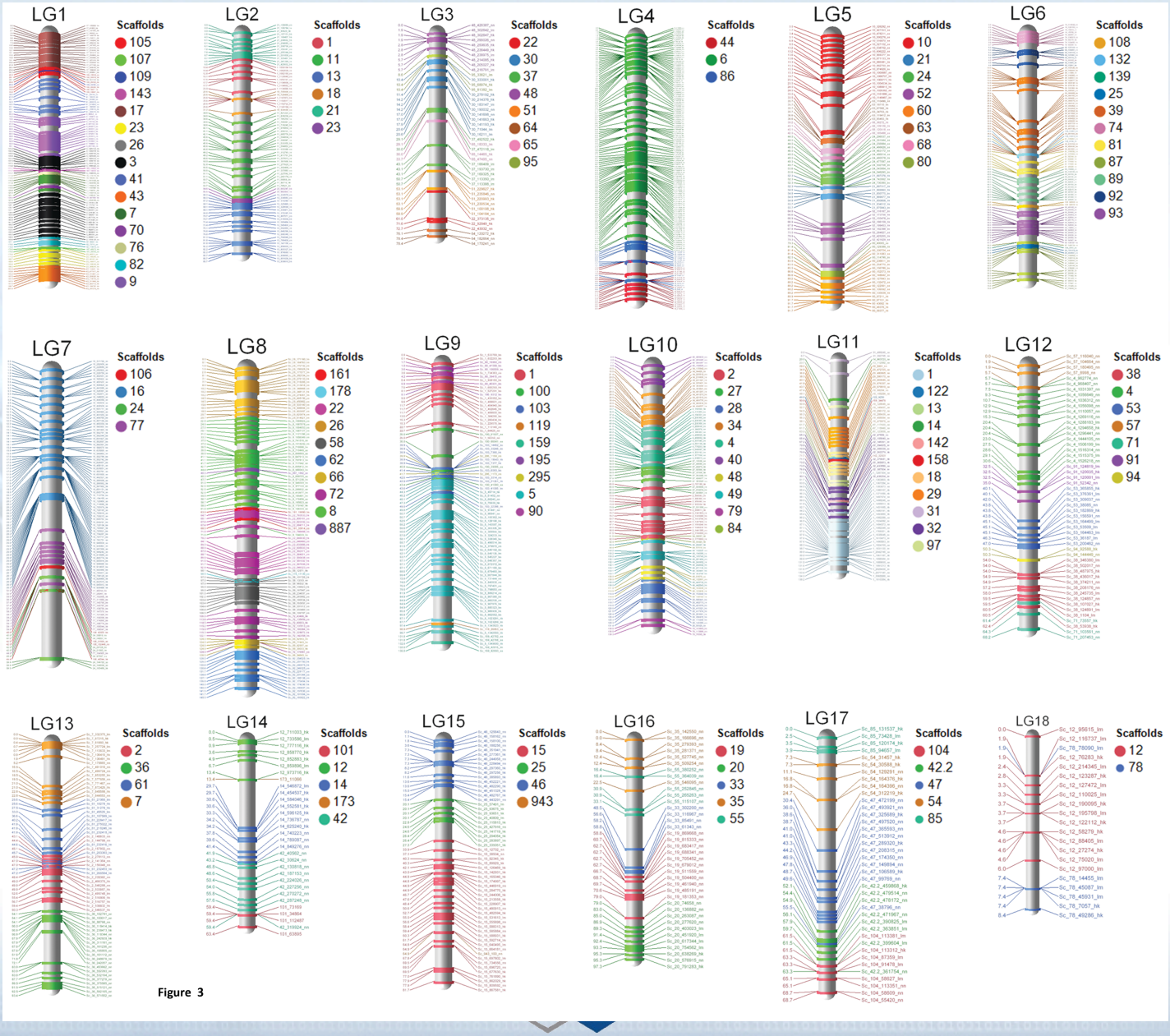


Figure 3

18 LGs

1654 cM

84% of assembly anchored

90% of genes anchored

LOH: skewed ratios and inexplicable genotypes

Sexual Recombination		Loss of Heterozygosity
AA x AA	→	**AA**
BB x BB	→	**BB**
AA x BB	→	**AB**
AB x AA	→	**AA, AB (1:1)**
AB x BB	→	**AB, BB (1:1)**
AB x AB	→	**AA, AB, BB (1:2:1)**

Asexual Reproduction		
AA	→	**AA**
BB	→	**BB**
AB	→	**AB**

Some obvious alternate explanations

- Base calling/genotyping errors
- Heterozygous restriction site

Both of these explanations result in sporadic and (presumably) random base call changes while LOH results in long continuous stretches that span multiple sequencing islands and, often, scaffolds.

No evidence for reduced coverage that would result from heterozygous restriction sites

Sequencing additional isolates (full genome sequencing)

Loss of Heterozygosity

- Must span at least two sequenced regions (RAD tags)
- Start and stop at unambiguous “LOH” loci
  - AAxBB -> AB progeny

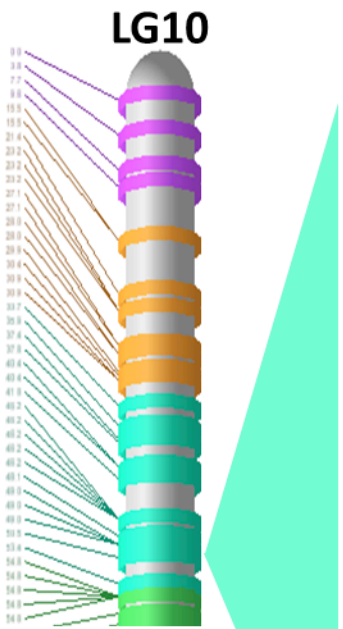
Impact of LOH

- Tracts ranged from 300bp to >1 Mbp
- LOH in 23 (>1/3) sexual progeny and 2 of the three clonal lineages.
- 14 of the 18 linkage groups.
- Directly impacted 11,048 SNPs (>1/2)
- Spanned 20Mbp (31%) of the reference genome.

Functional impact

- LOH changed 391 normally segregating markers to homozygosity for amino acid changing substitutions that were not homozygous in either parent and would not be possible due to sexual recombination.
- Instant evolution

Mating Type Switch

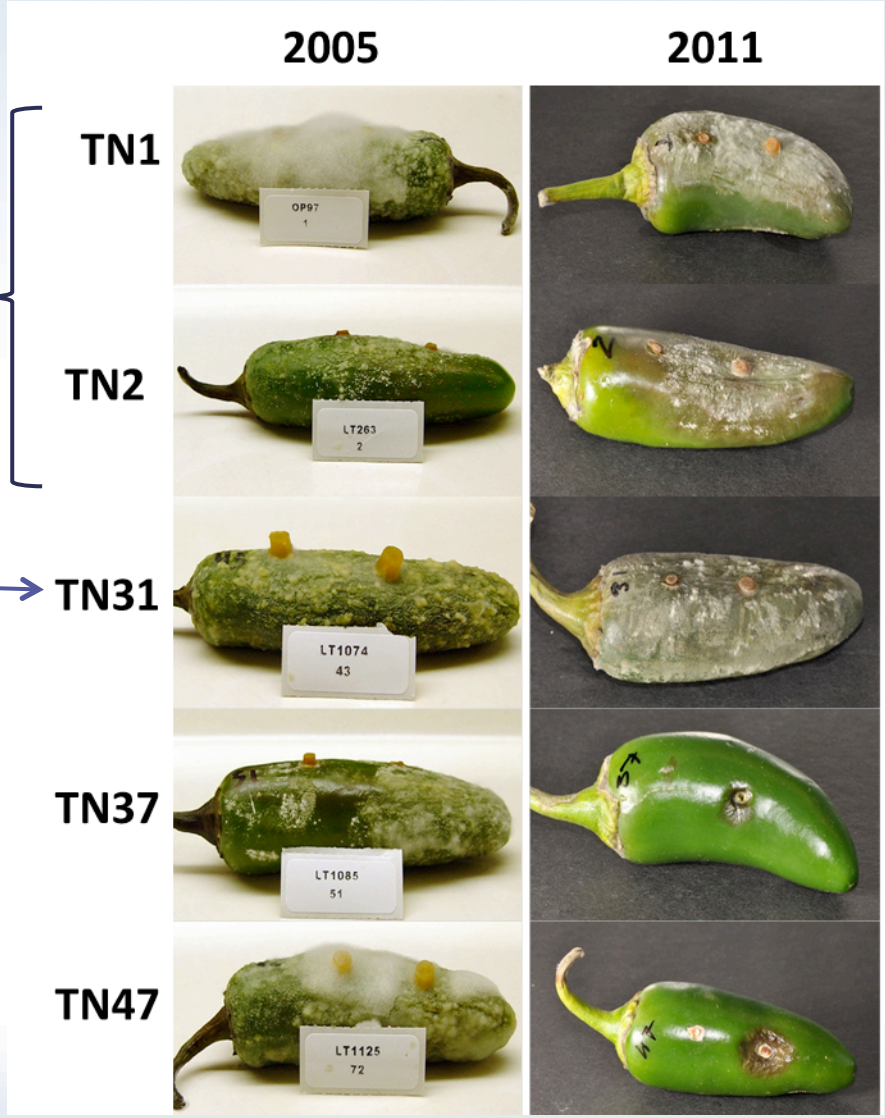


Isolate	TN1	Group 1	18, 34, 53, 63	47	52	6	32, 69	4	25	Group 2	TN2
LOH	No	No	Yes	Yes	Yes	No	No	No	No	No	No
Mating type 2005	A1	A1	A2	A1	A1	A2	A1	A2	A1	A2	A2
Mating type 2011	A1	A1	A1	A1	A1	A1	A1	A2	A2	A2	A2
* 4_875573	AA	RA	AA	RR	AA	RA	RA	RA	RA	RA	RR
4_877151	AA	RA	AA	RR	AA	AA	AA	AA	AA	AA	RA
* 4_877466	AA	RA	AA	RR	AA	RA	RA	RA	RA	RA	RR
4_877504	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
* 4_880664	AA	RA	AA	RR	AA	RA	RA	RA	RA	RA	RR
4_880689	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_880712	RR	RR	RR	RR	RR	.	RA	RA	RA	RA	RA
4_881577	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_881580	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_881607	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_881629	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_881656	AA	RA	AA	RR	AA	AA	AA	AA	AA	AA	RA
4_882535	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_882547	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_882548	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA	RA
4_883297	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA	RA
4_883314	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA	RA
4_883328	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA	RA
4_883362	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA	RA
4_897805	AA	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA
4_897880	AA	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA
4_898549	AA	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA
4_899846	AA	AA	AA	AA	AA	AA	RA	RA	RA	RA	RA
4_901140	RR	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA
4_901147	RR	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA
4_902744	RR	RR	RR	RR	RR	RR	RA	RA	RA	RA	RA
* 4_907338	AA	RA	AA	RR	AA	RA	RA	RA	RA	RA	RR
* 4_907371	AA	RA	AA	RR	AA	RA	RA	RA	RA	RA	RR
4_908701	AA	AA	AA	.	AA	AA	RA	AA	RA	RA	RA

Pathogenicity

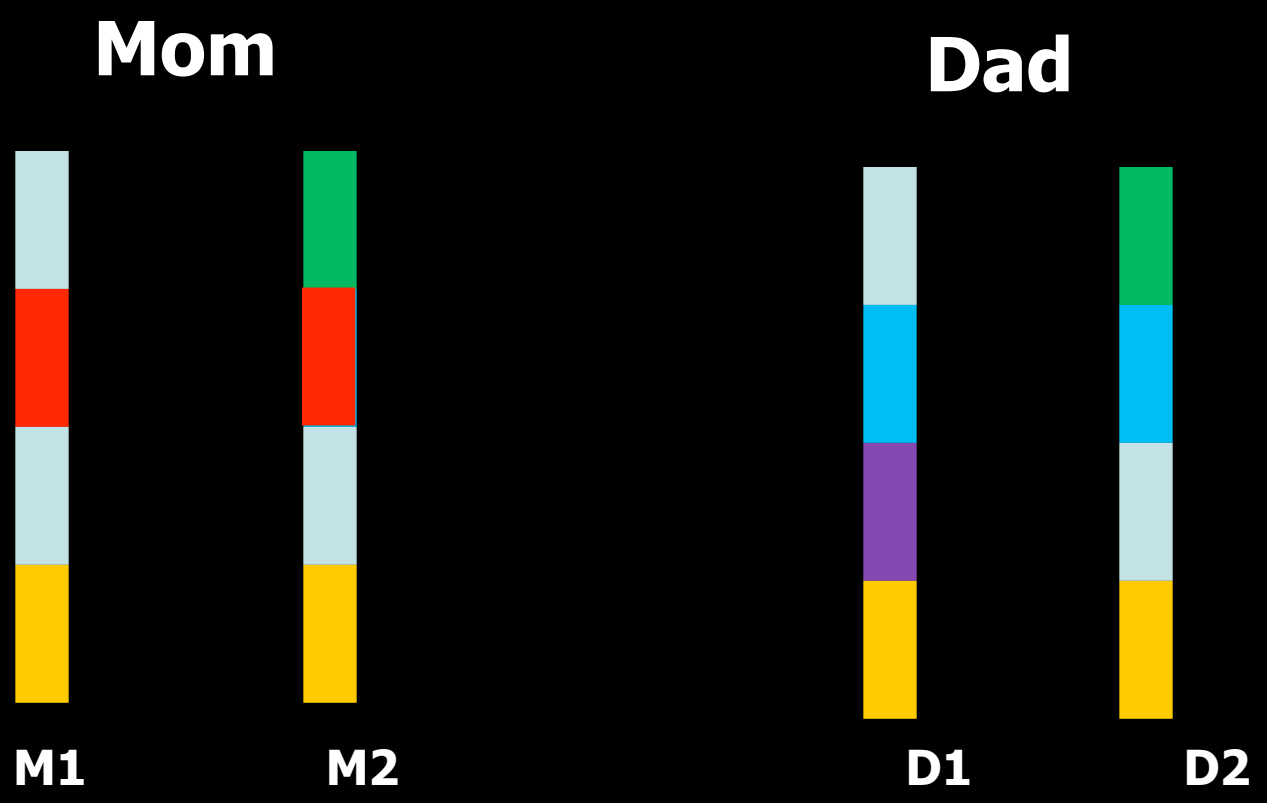
Parents

Typical of all progeny except the two below



TN37 and TN47 had the most LOH of any isolate (both >5Mb and >10%).

Red block = Ridomil Resistance



One copy = ½ resistance, two copies = full resistance

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Examples of LOH in other systems

- *Phytophthora colocasiae, Phytophthora ramorum, Saprolegnia parasitica*
- *Candida albicans*
  - stress-induced adaptive evolution
    - fluconazole resistance
  - white-opaque switching
- *Homo sapiens*
  - oncogenesis
  - embryonic stem cell adaptation

Forche et al, 2011
Morschhäuser 2010
Narva et al., 2010
Rosenberg 2011

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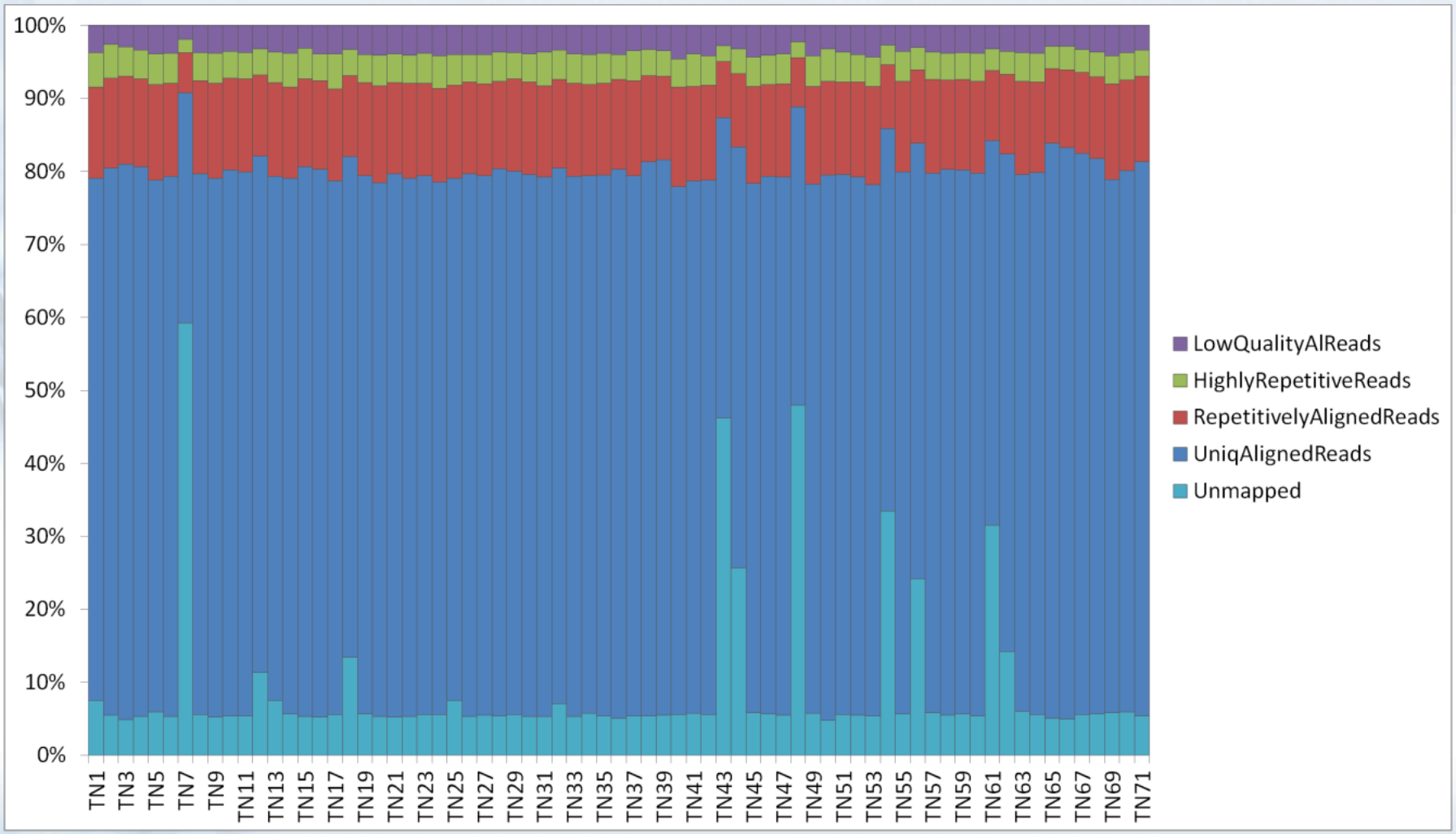
Reference Genome

- **Based on 454 (30X) + Sanger (5X)**

Assembly	64 Mbp
Scaffolds	917
Scaffold N50	706 kb
Genes	17,123

http://genome.jgi-psf.org/Phyca11/Phyca11.home.html

Proportion of reads aligning



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Mating Type Switch in *Candida albicans*

Morcshhäuser, J. 2010. Med. Microbial Immunol. 199:165-72.

- Diploid fungus
- "White Opaque Switching"
  - White=yeast morphology, more virulent
  - Opaque=elongated, mating competent
  - MTL locus
    - Heterozygotes: heterodimeric repressor of Wor1, which is required for mating competence
    - LOH at the MTL locus allows the switch to mating competence.