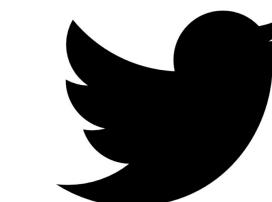


Genetic basis of traits in the fungal pathogen of wheat *Zymoseptoria tritici*



LabMeeting 18.11.21

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@jessstapley

Fungus septoria leaf blotch (STB)

Fungal pathogen *Zymoseptoria tritici*



Zymoseptoria tritici: a devastating wheat pathogen

Z. tritici causal agent of septoria tritici leaf blotch (STB)

- Worldwide distribution
- Up to 50% yield loss - untreated
- UK, France and Germany - direct costs of yield loss €120-700 million and cost of Fungicide treatment €160-500 million*

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Ensure our future food Security!

- 60% more grain by 2050

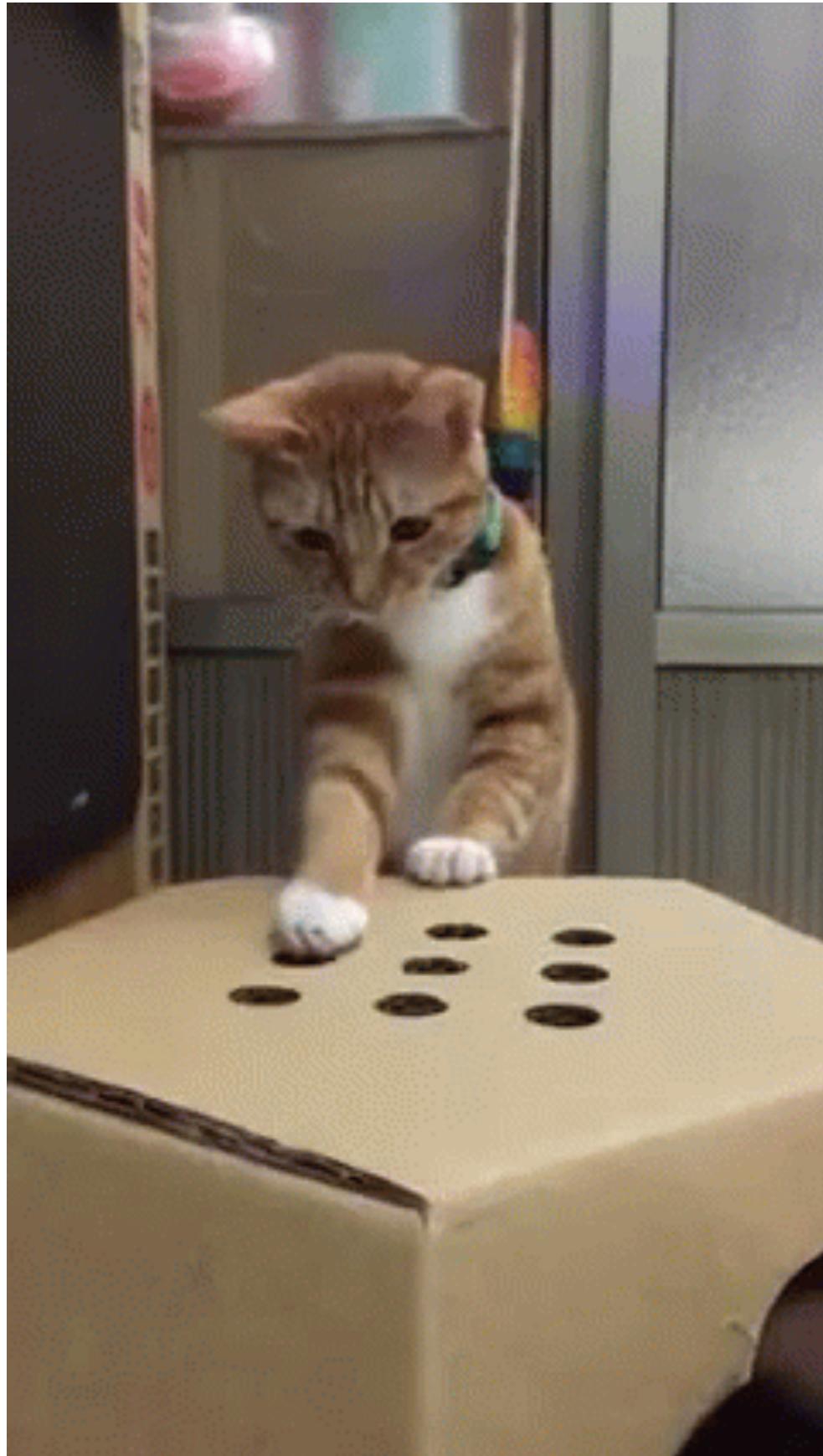


FEED

Switzerland's iconic foods at risk



Controlling *Zymoseptoria tritici* is like playing whack-a-mole



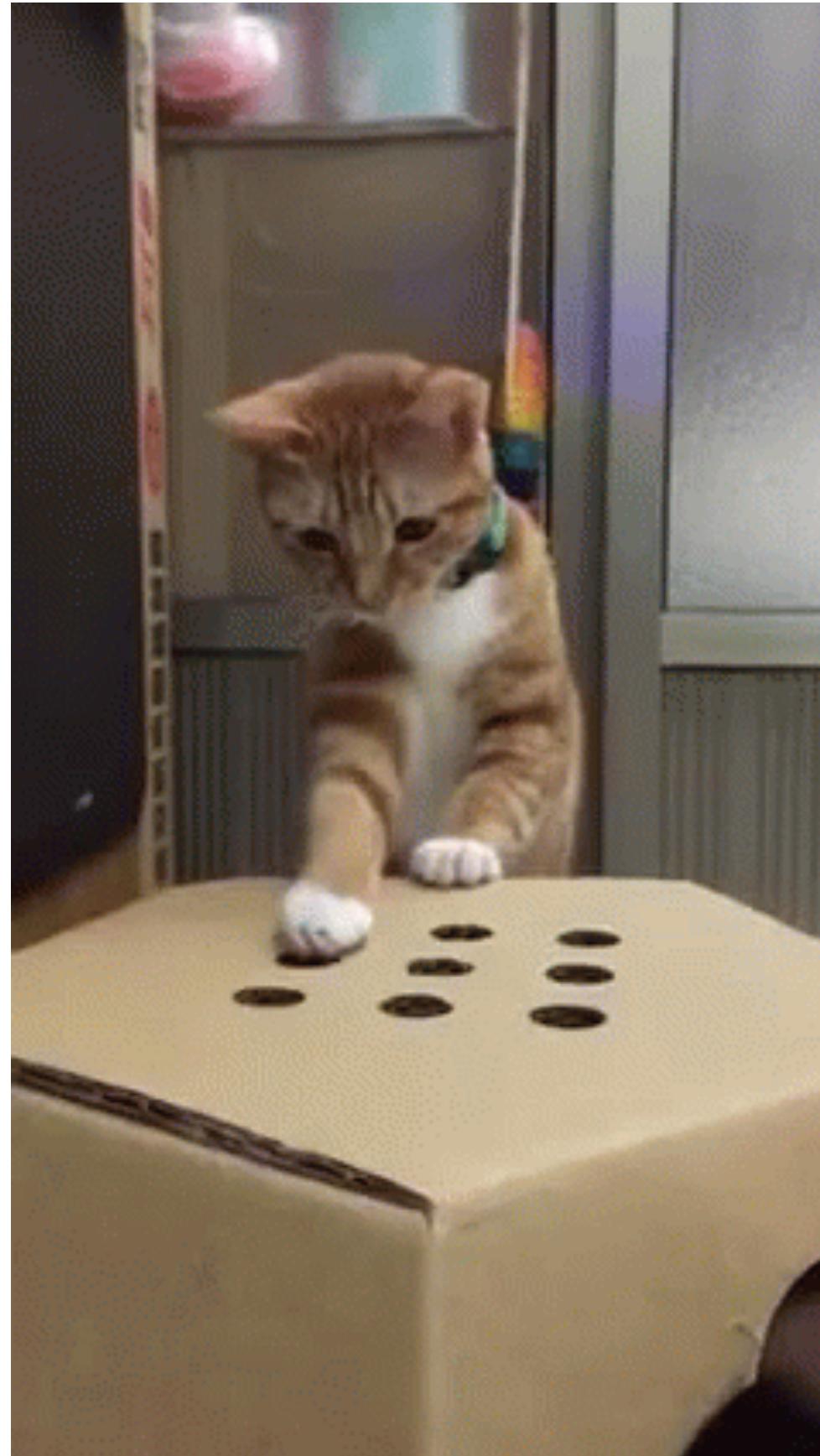
Rapid evolution of the pathogen to fungicides and resistant cultivars

Whack-a-mole

“a situation characterized by a series of repetitious and futile tasks, where the successful completion of one just yields another popping up elsewhere”

Development of STB resistant wheat cultivars

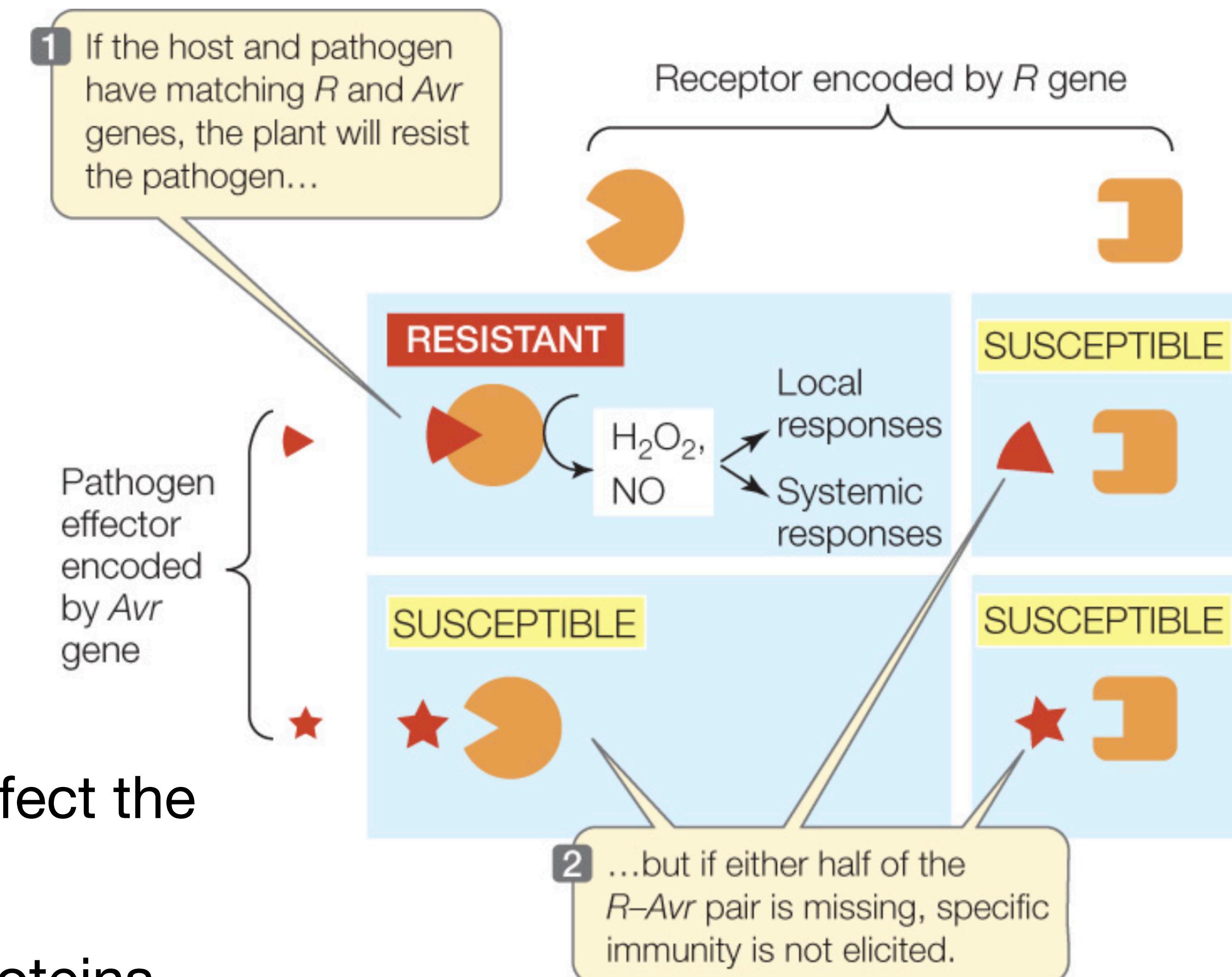
STB resistance can be quantitative and or qualitative



- **qualitative**
 - one or few large effect loci
 - gene-for-gene - fungal avirulence (Avr) proteins interact with a specific plant resistant (R) protein

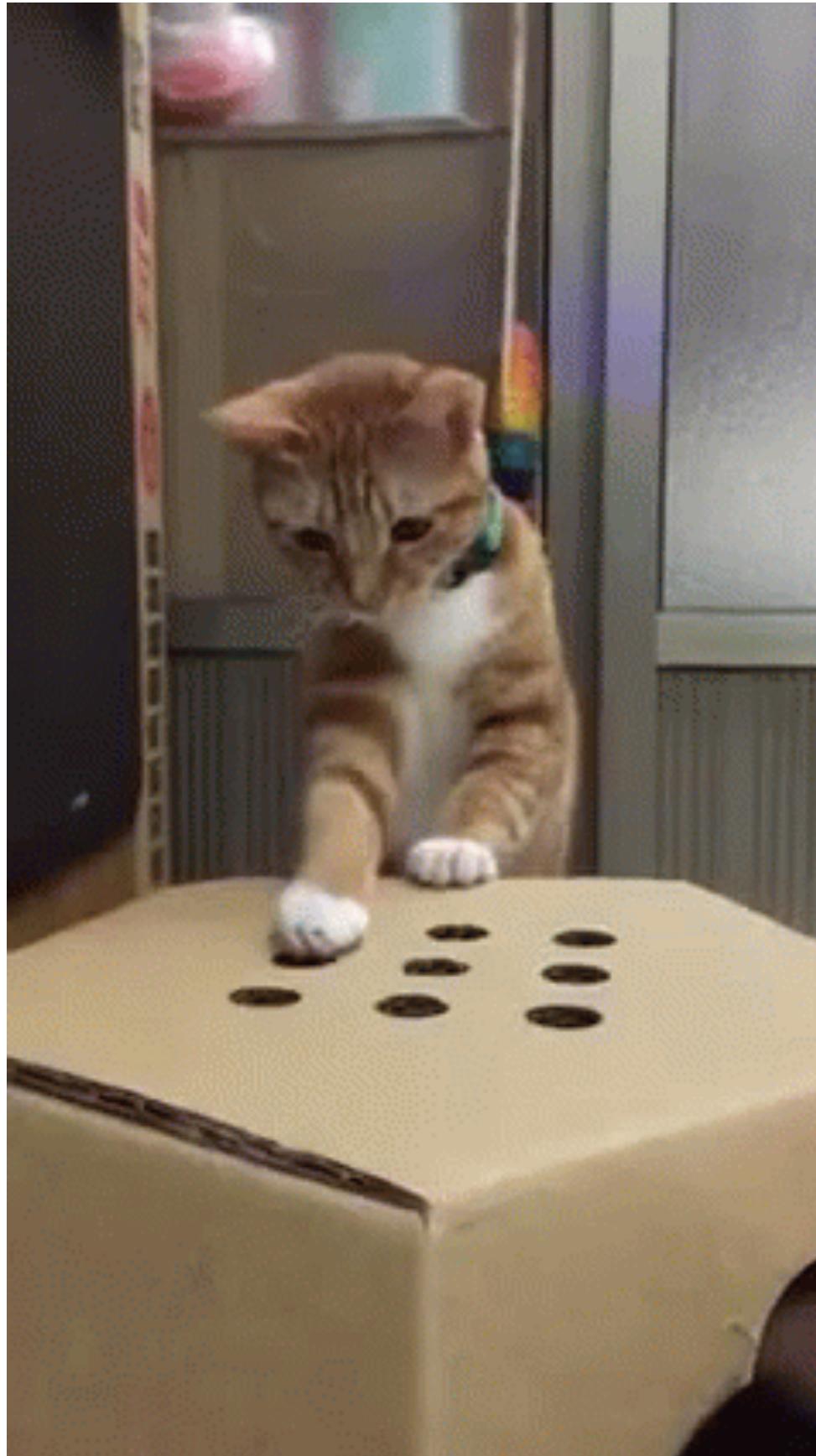
Gene-for-gene model

- type of **qualitative** resistance
- when plants detect the fungus they mount an immune response and kill it
- plant has a resistance protein (*R*) that can recognise a fungal protein (*Avr*)
- specific pairs of genes must match
- **Boom-bust-cycles**
 - fungus evolves new *Avr* protein to infect the host - fungal population goes boom
 - the plant evolves new recognition proteins - fungal population goes bust



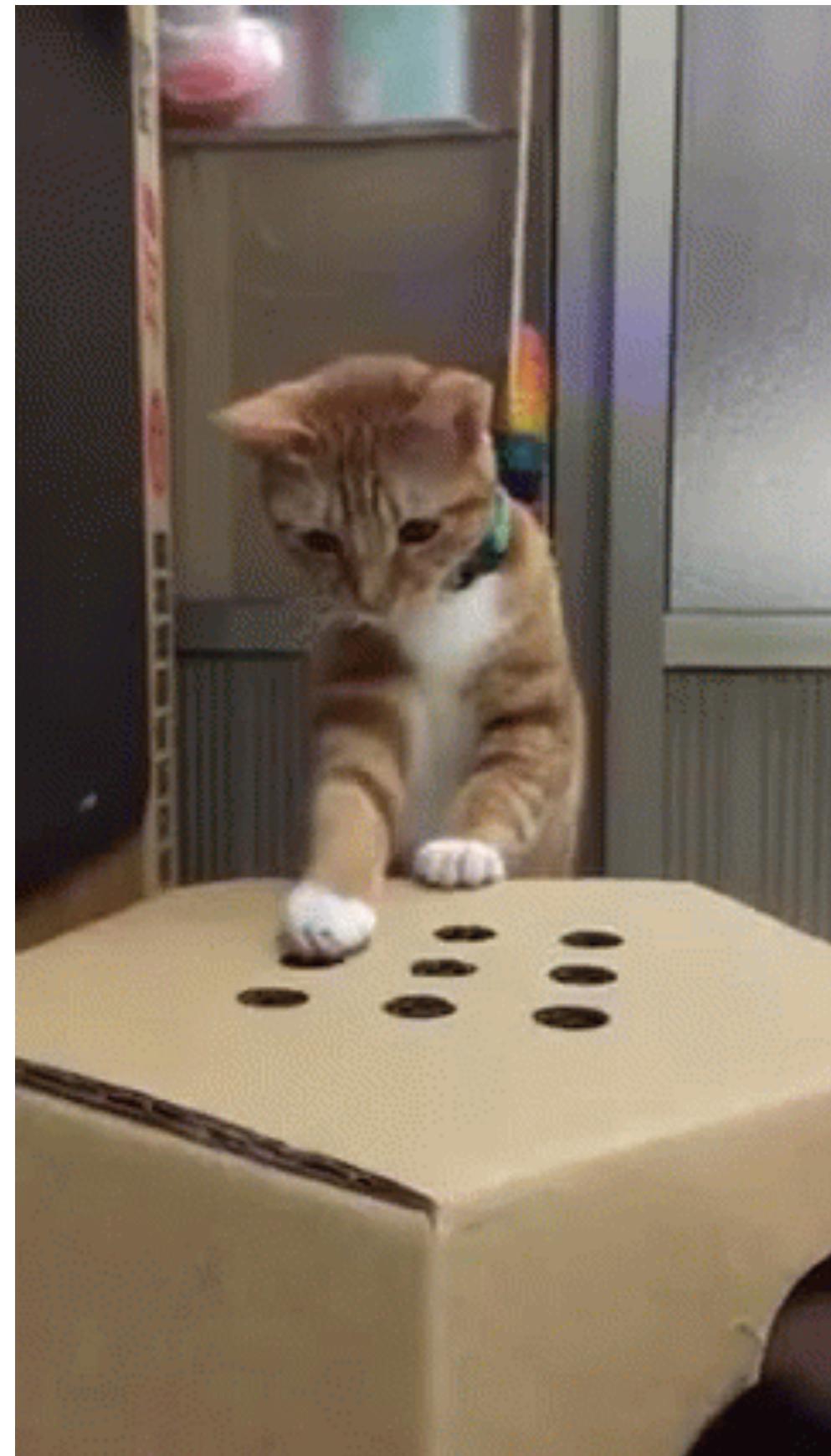
Development of STB resistant wheat cultivars

STB resistance can be quantitative and or qualitative



- **qualitative**
 - one or few large effect loci
 - gene-for-gene - fungal avirulence (Avr) proteins interact with a specific plant resistant (R) protein
 - only effective against avirulent Zt genotypes
- **quantitative**
 - many genomic regions (loci) and 00-000s genes
 - effective against multiple Zt genotypes/isolates

Zymo adaption to resistant wheat cultivars



- **qualitative - not durable** X
- Europe - all known resistant genes are no longer effective against *Z. tritici*

- **quantitative - durable** ✓
- *slower to erode, partial resistance*

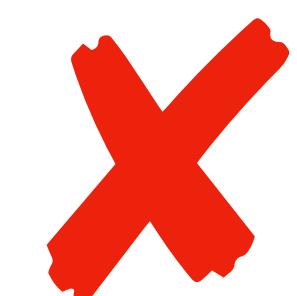
Zymo adaptation to Fungicides



- 1970s Benzimidazoles - no longer used, fungal resistant mutation is widespread

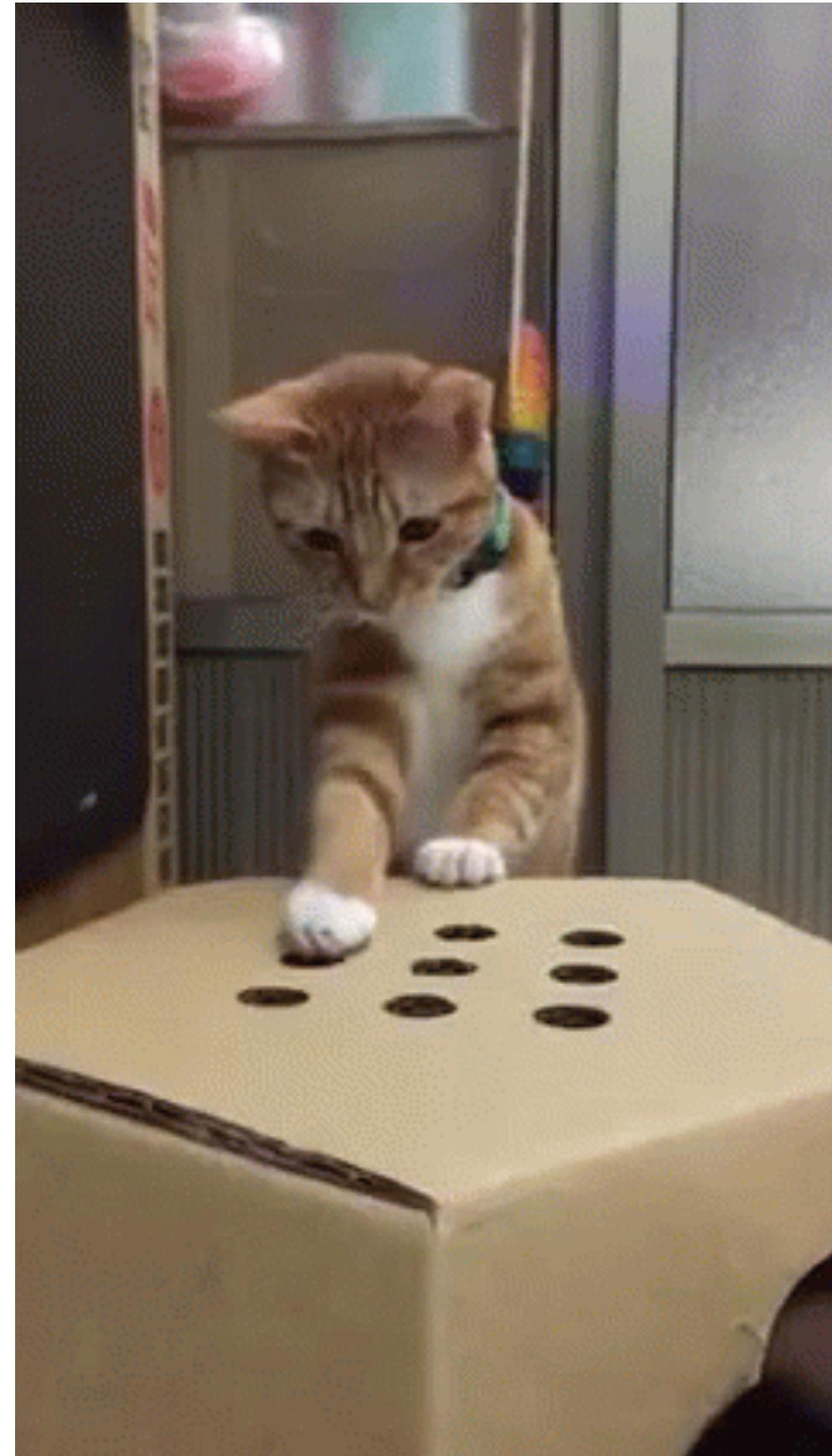


- 1970s DMI/Azole - still effective, despite multiple resistance mutations, resistance is slow because trait is **quantitative**



- 1990s Qols/strobilurins - almost all *Z. tritici* have the resistant alleles

Controlling *Zymoseptoria tritici* is like playing whack-a-mole



Rapid evolution of the pathogen to fungicides and resistant cultivars

Rapid adaptation of the fungus to

- wheat cultivars with qualitative resistance
- fungicides that can be resisted with a major gene

Adaptation to wheat cultivars with quantitative resistance is slower. Fungicide adaption that

Quantitative genetics studies of *Z. tritici*

Knowledge of how *Z. tritici* escapes control methods can have both economical and environmental benefits

- need novel modes of actions and molecules involved in plant-pathogen and plant-fungicide interactions

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Genetic Mapping at ETH Plant Pathology Group



Understanding pathogen ecology and evolution using state-of-the-art techniques

- High Throughput Digital Phenotyping
- Whole genome sequencing
- High throughput genotyping

Develop effective and sustainable pathogen control strategies

Genetic mapping of traits in *Z. tritici*

Identify genomic regions (loci) and genes controlling *Z. tritici* traits

- Fungicide resistance
- Virulence
- Growth and reproduction
- Response to the environment (stress response) - influence
 - interactions between plant and pathogen
 - genetic variation and local adaptation

Finding genomic regions and genes

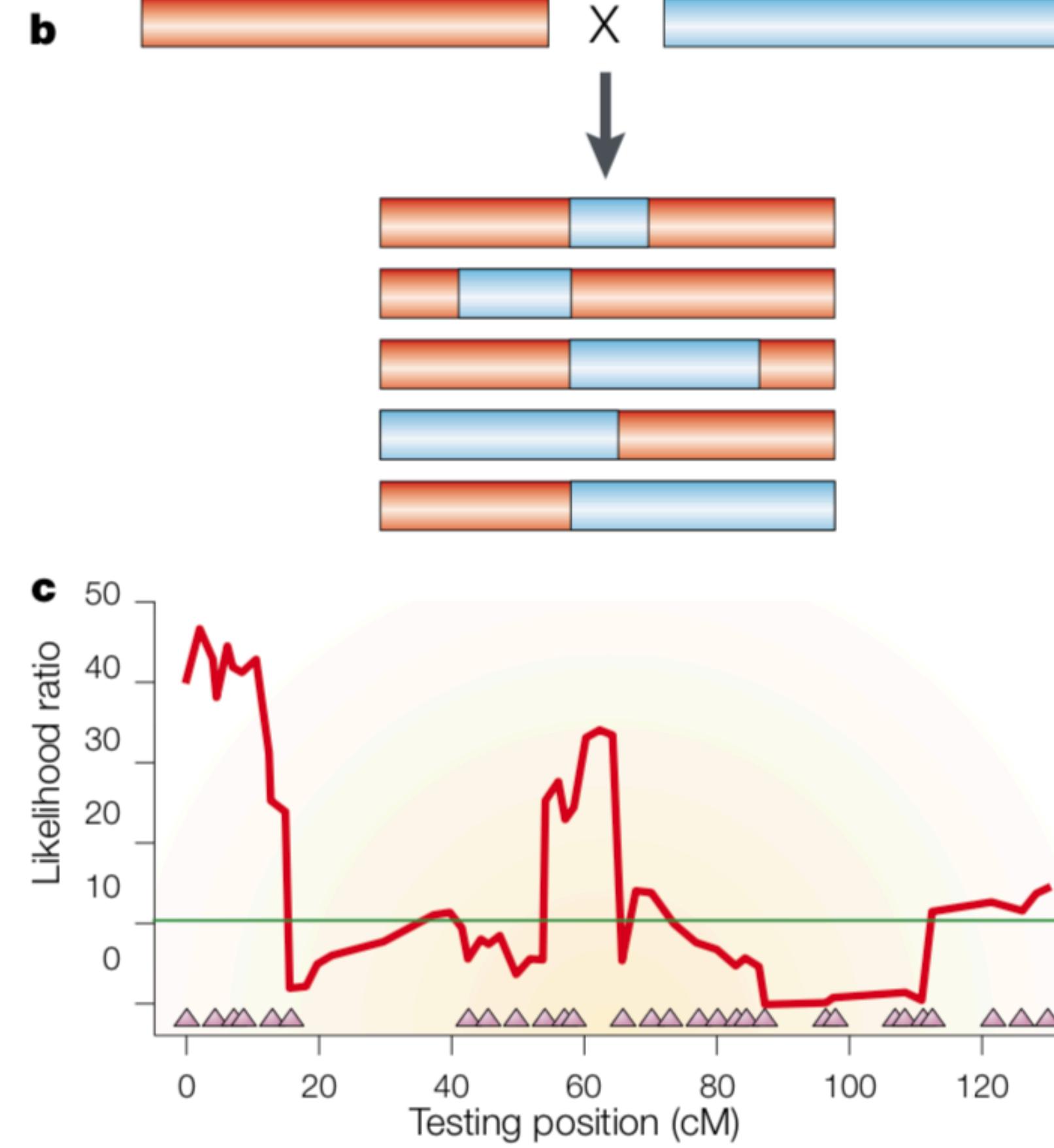
Quantitive trait loci (QTL) mapping and genome-wide association scans (GWAS)

QTL: A Quantitative Trait Loci is a region on a chromosome (a locus) where variation in the sequence of DNA is statistically associated with variation in the phenotypic trait.

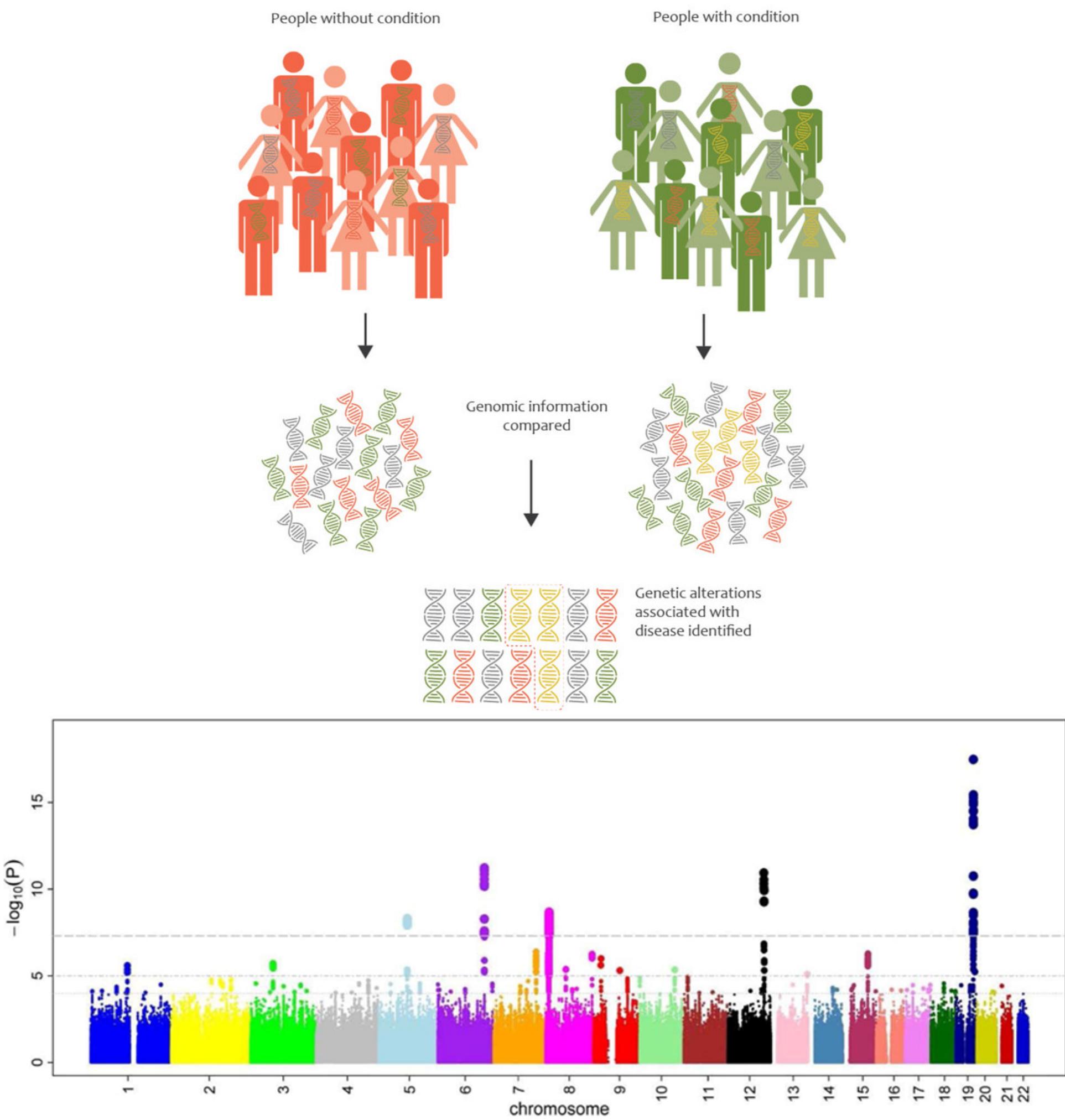
GWAS: Genome Wide Association Study. Identify a statistical association between genetic variation and phenotypic variation

Z. tritici QTL mapping and GWAS

QTL mapping



GWAS



Z. tritici QTL mapping

Crosses were made between parents collected in the field in Switzerland 1999

- Leaves were co-infected with parental strains, the resulting ascospores were collected, grown in vitro and stored at -80°C
 - 3D7 x 3D1, 359 offspring
 - 1A5 x 1E4 - 341 offspring

RAD tag sequencing was used to genotype offspring

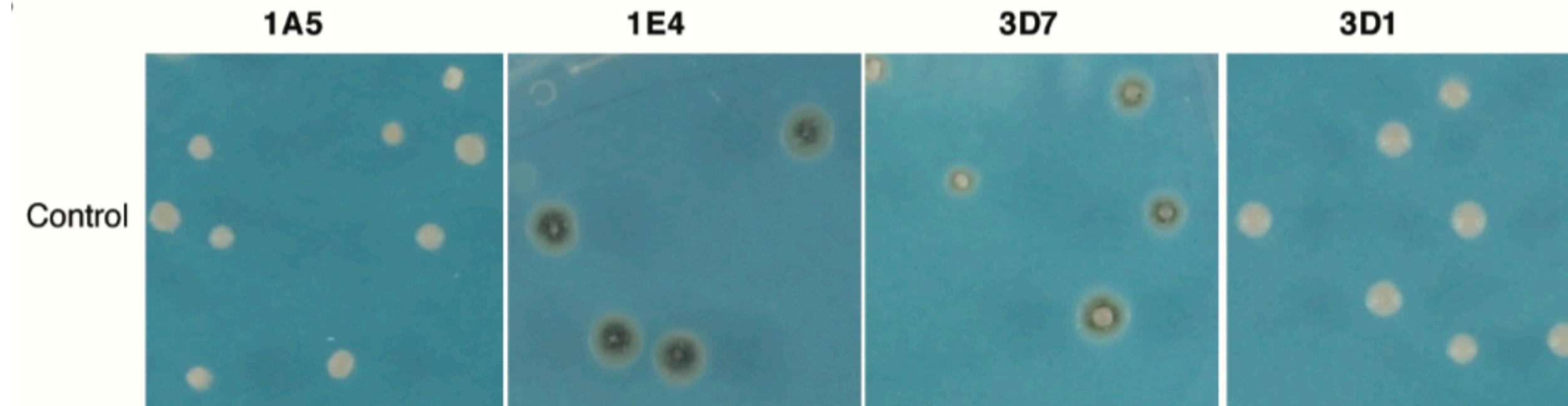
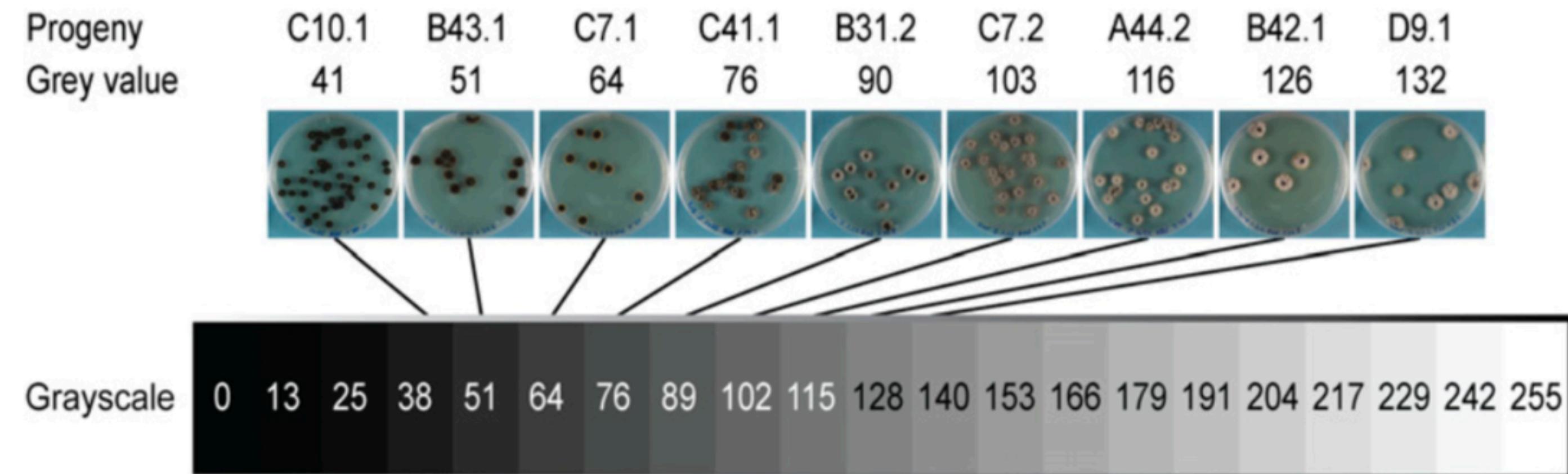


Photo: Zhong PhD thesis, pg 67

In vitro (on plate): Size and melanin

- photograph at multiple time points: 8, 11, 12 14 days post inoculation (dpi)
 - measured two traits:
 - 1) mean colony area - converted to radial size
 - 2) mean grey value - measure of cell melanisation

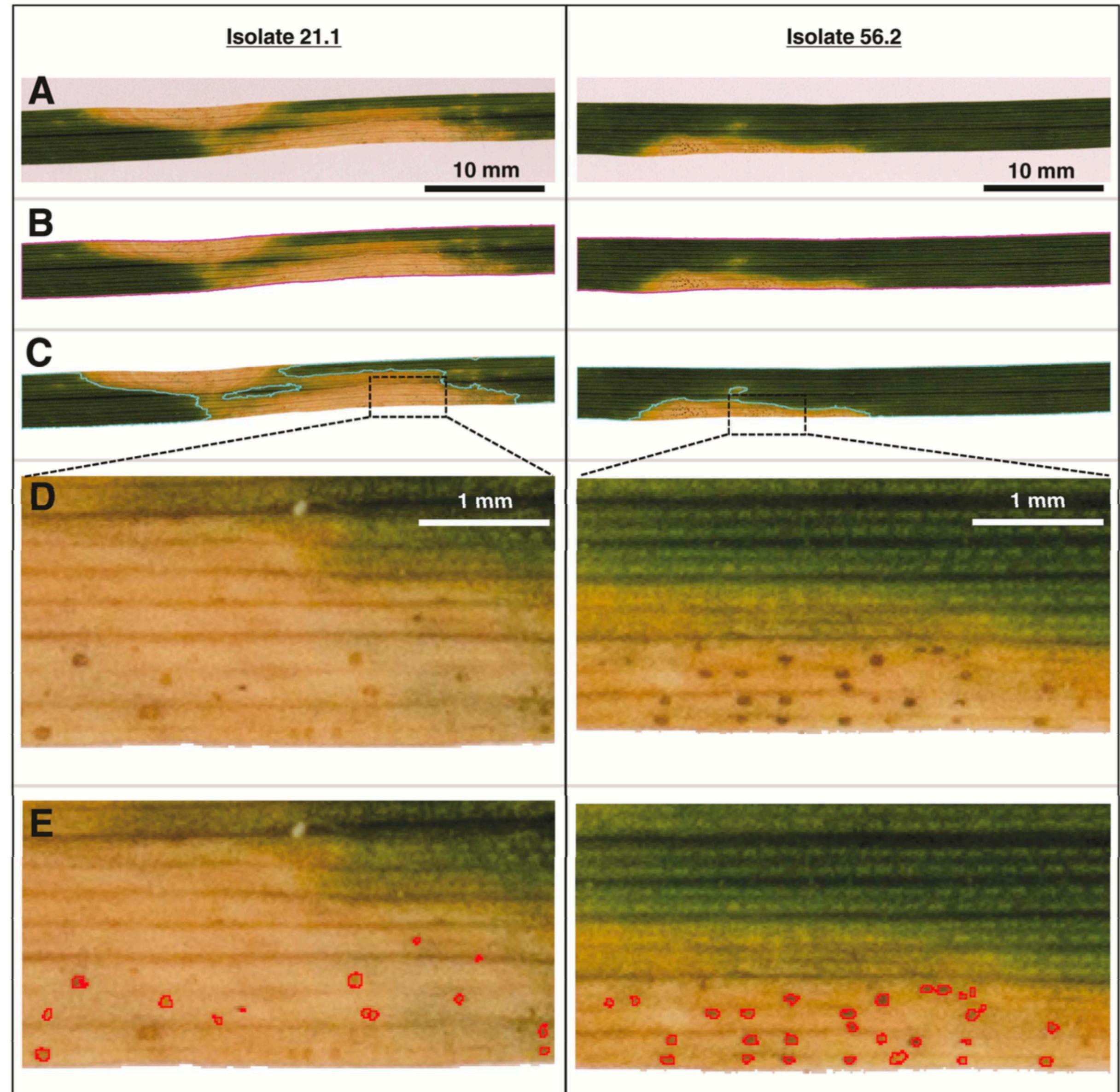


$$\text{size} = \sqrt{\frac{\text{area}}{\pi}}$$

** Grey value inversely related to melanin

In vivo (on plant): Lesion size, pycnidia

- Lesion size and pycnidia count
 - Pycnidia: reproductive fruiting bodies
 - Pycnidia density
 - Pycnidia grey value (melanin)



Example gene mapping studies

1. QTL mapping of Fungicide sensitivity

- Lendenmann et al 2014

QTL mapping of fungicide sensitivity reveals novel genes and pleiotropy with melanization in the pathogen *Zymoseptoria tritici*

Mark H. Lendenmann *, Daniel Croll, Bruce A. McDonald

Institute of Integrative Biology, Plant Pathology Group, ETH Zürich, Universitätstrasse 2, 8092 Zürich, Switzerland

2. QTL, GWAS and validation of avirulence (Avr) gene

- Zhong et al 2017



Research

A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene

3. QTL mapping of osmotic stress response

- Stapley et al (in prep)

Ziming Zhong^{1*}, Thierry C. Marcel^{2*}, Fanny E. Hartmann¹, Xin Ma¹, Clémence Plissonneau^{1,2}, Marcello Zala¹, Aurélie Ducasse², Johann Confais², Jérôme Compain³, Nicolas Lapalu^{2,3}, Joëlle Amselem³, Bruce A. McDonald¹, Daniel Croll^{1,4†} and Javier Palma-Guerrero^{1†}

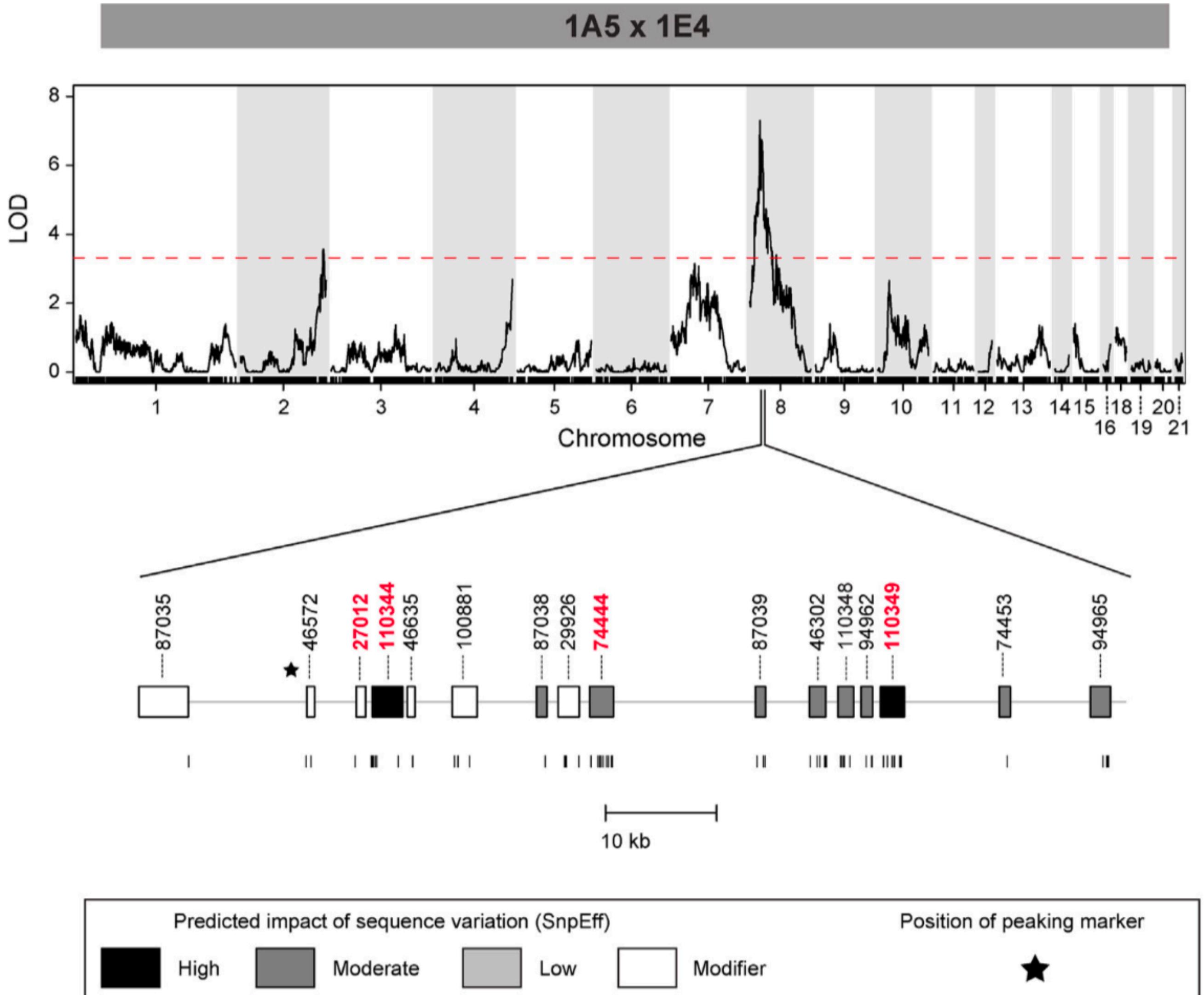
¹Plant Pathology Group, ETH Zürich, Universitätstrasse 2, 8092 Zürich, Switzerland; ²UMR BIOGER, INRA, AgroParisTech, Université Paris-Saclay, 78850 Thiverval-Grignon, France; ³UR URGI, INRA, Université Paris-Saclay, 78026 Versailles, France; ⁴Laboratory of Evolutionary Genetics, University of Neuchâtel Institute of Biology Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland

QTL mapping of fungicide sensitivity

- **Trade-off between growth and fungicide sensitivity**
 - faster growth - greater fungicide sensitivity
- **QTLs for growth in both environments**
 - presence and absence of fungicide - genes that are important for growth in a benign environment are also important for growth in presence of fungicide
- **QTLs for fungicide sensitivity**
 - find candidate genes for fungicide sensitivity

QTL mapping of fungicide sensitivity

M.H. Lendenmann et al./Fungal Genetics and Biology 80 (2015) 53–67



QTL on Chromosome 8

QTL interval contained 16 genes that are effected by sequence variation

Genes in red - good candidate genes based the sequence variation between parents

Gene 27012 is involved in membrane transport

Example gene mapping studies

1. QTL mapping of Fungicide sensitivity

- Identified novel candidate genes for fungicide sensitivity

2. QTL, GWAS and validation of avirulence (Avr) gene

- Zhong et al 2017

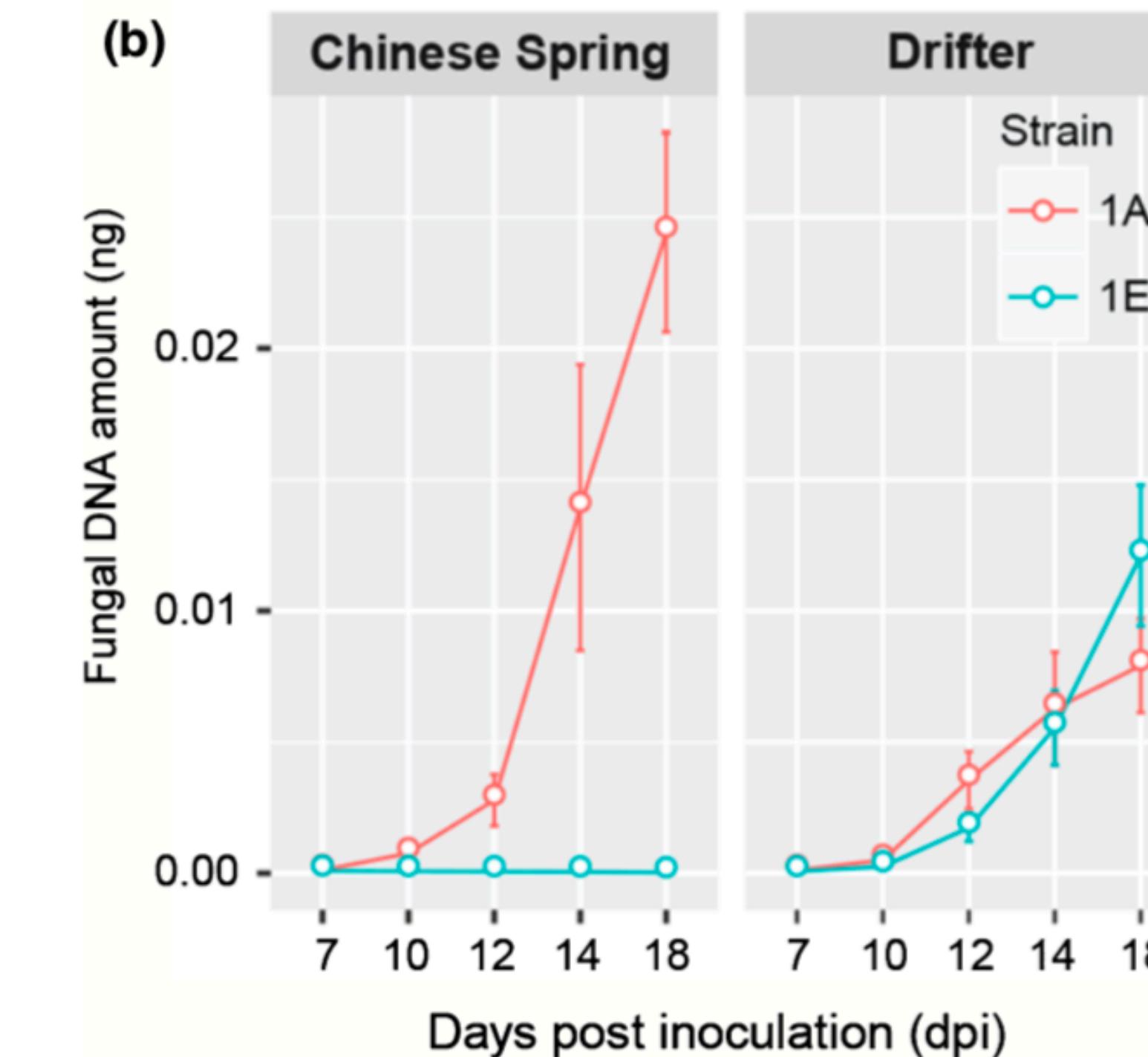
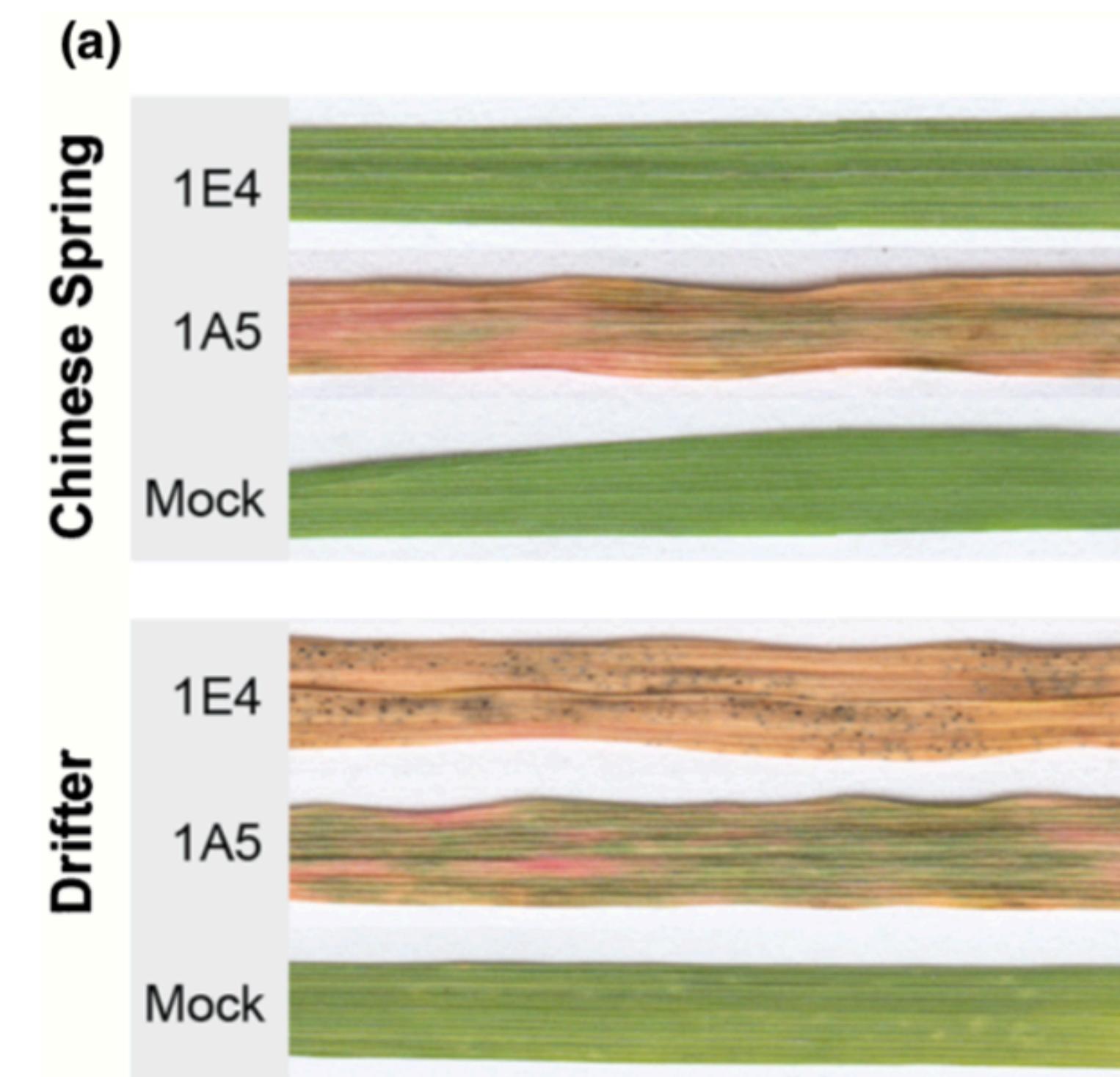
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Mapping and validation of Avr genes in *Z. tritici*

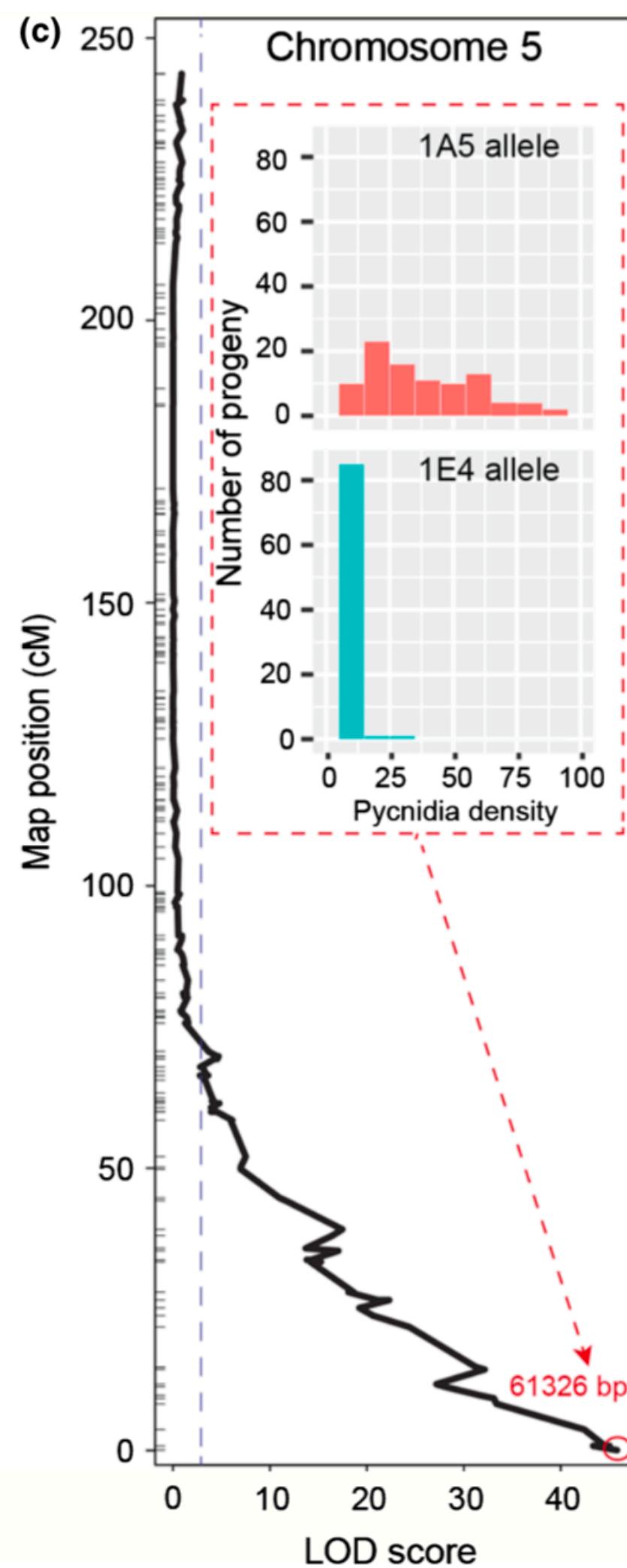
Two wheat cultivars - Chinese spring has the resistance gene Stb6, Drifter lacking the resistance gene Stb6

- 1E4 is virulent on Chinese spring - does it have AvrStb6 gene



QTL and GWAS identify same gene

QTL on Chromosome 5



QTL - nine genes in QTL interval

Two chosen as candidates

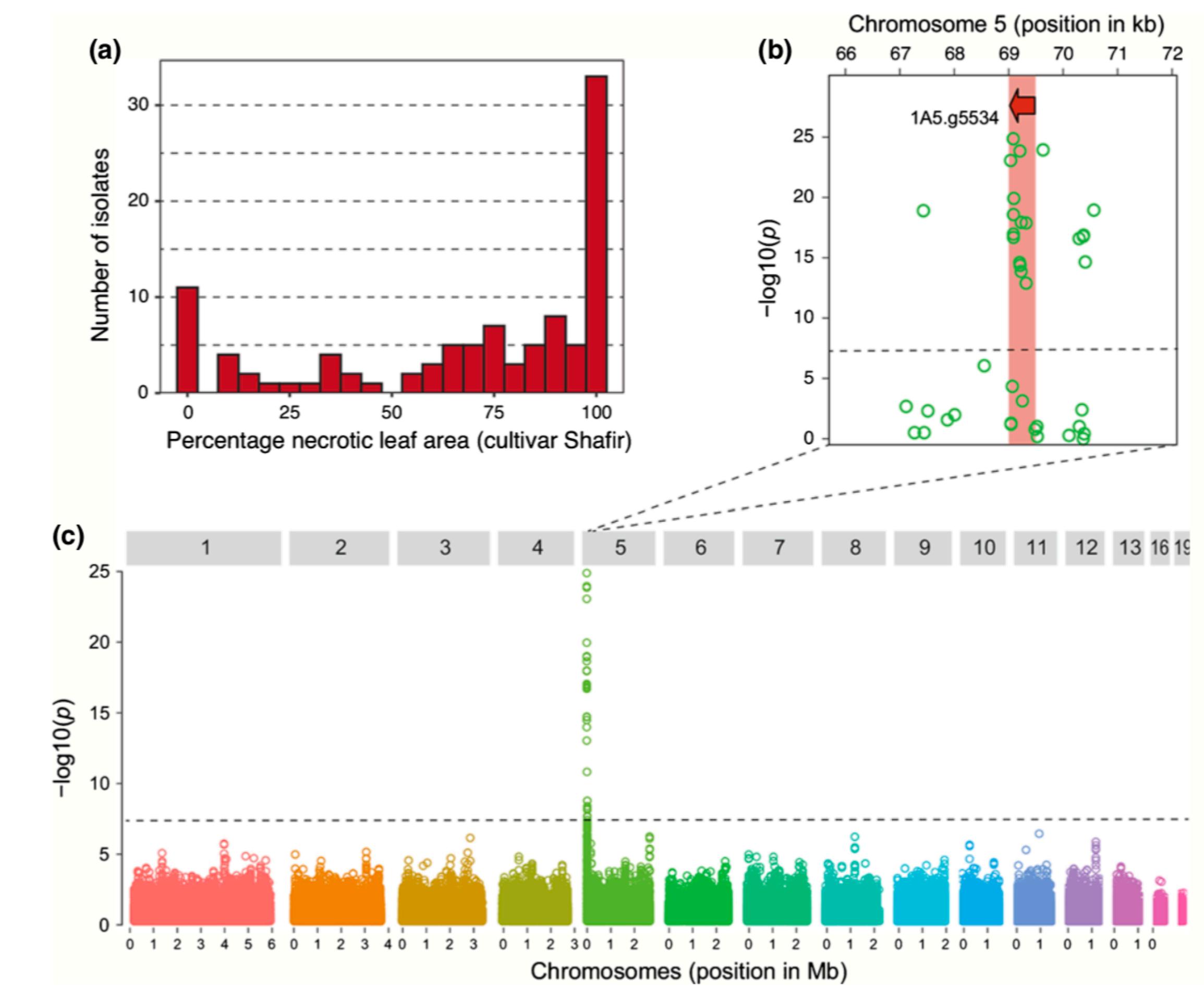
- sequence variation
- effector protein

GWAS - some isolates virulent on wheat with Stb6

Sig. SNPs within open reading frame of 1A5.g5534

Gene functionally annotated

GWAS - 103 isolates from France



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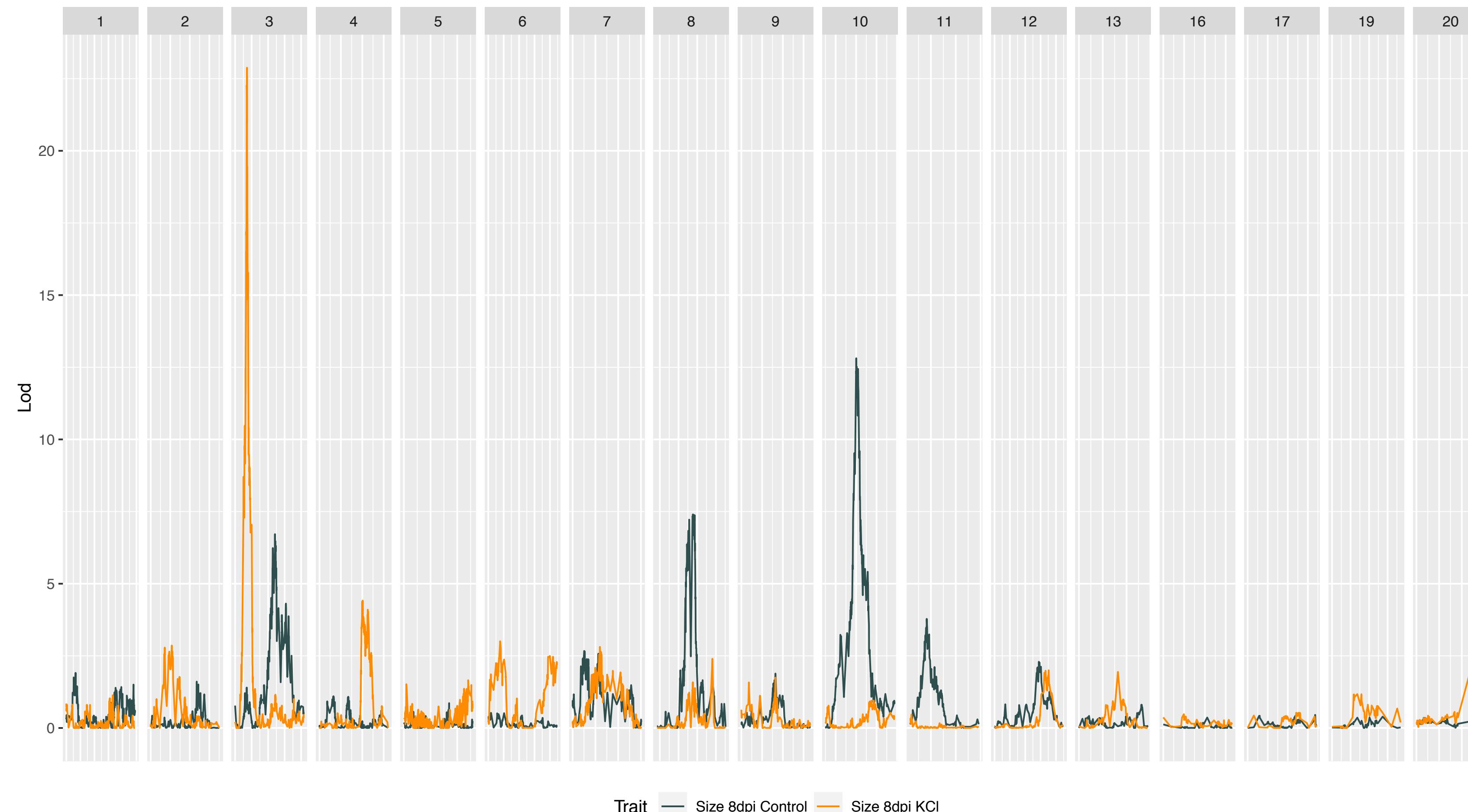
- Identified the first AvrStb6 gene

3. QTL mapping of osmotic stress response

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QTL for size at 8 dpi in KCl

3D7x3D1



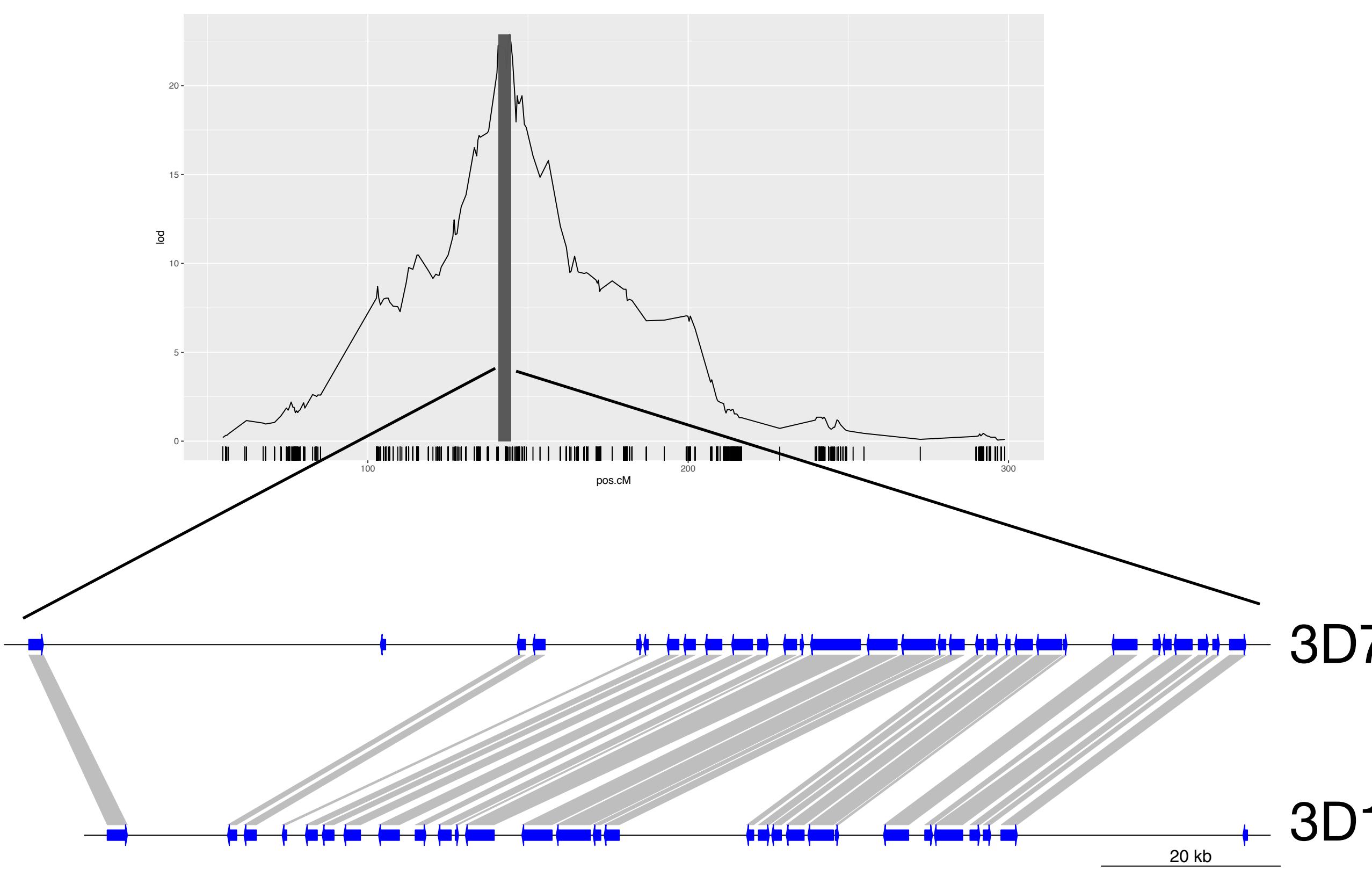
Size at 8 dpi mapped to a unique QTL with high LOD on Chr 3

Interval contained 94 SNPs and 31 genes

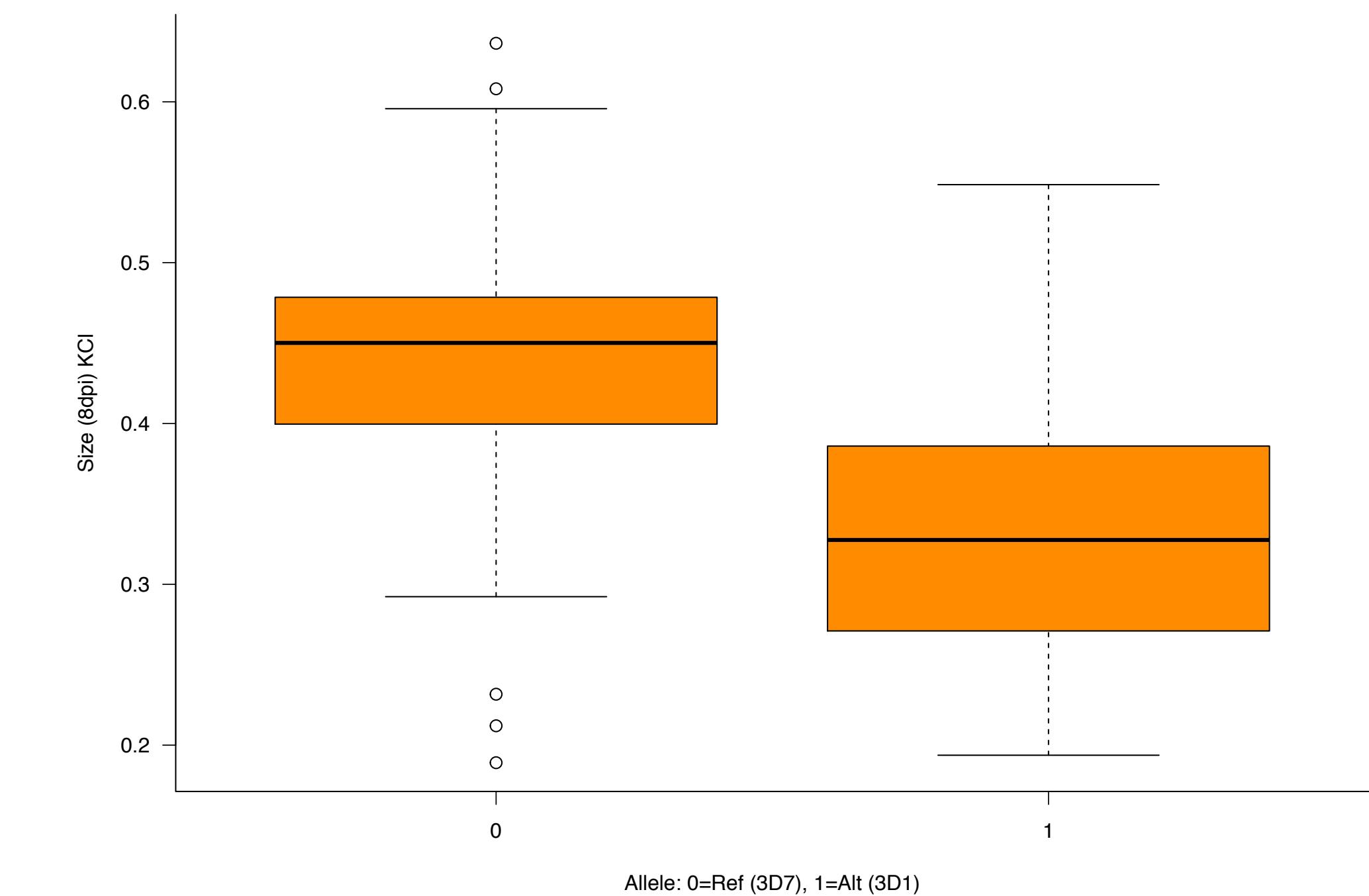
PVE = 0.33

QTL for size at 8 dpi in KCI

LOD Peak and BCI Interval (shaded)

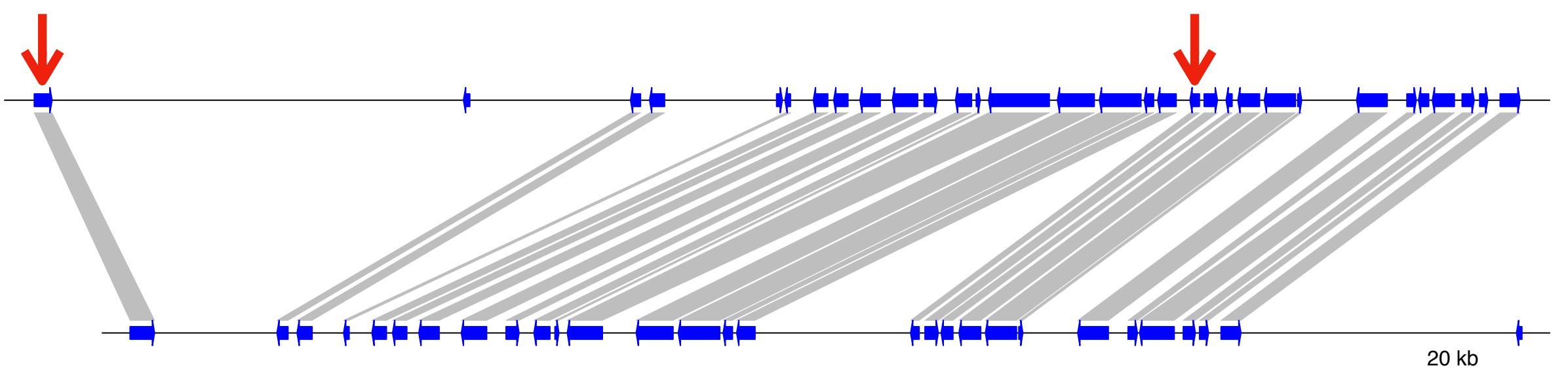


SNP with highest LOD - isolates with 3D1 allele were larger than isolates with 3D1 allele



Two potential candidate genes

- ZT3D7_G3622 Fungal Specific Transcription Factor domain
 - Non synonymous SNPs in gene
- ZT3D7_G3639 Major Facilitator Superfamily
 - 8 bp deletion in gene



Gene	Protein Length	Gene Seq. Similarity	DGE	Secreted protein	Putative function
ZT3D7_G3622	550	97.4	In vitro: Down in mm	No	Fungal specific transcription factor domain
ZT3D7_G3639	512	98.66	In vivo: Down 12, 14 dpi	Yes	Major Facilitator Superfamily

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 - Identified novel candidate genes for fungicide sensitivity
2. QTL, GWAS and validation of avirulence (Avr) gene
 - Identified the first AvrStb6 gene
3. QTL mapping of osmotic stress response
 - Identified novel candidate genes for osmotic stress

Genetic mapping studies in *Z. tritici*

- first validated AvrStb2 gene - confirming the importance of gene-for-gene interactions and advancing our understanding of virulence
- identified candidate genes for fungicide resistance and osmotic stress, which could provide novel targets for new fungicides
- better understanding of fungal molecular biology and evolution will lead to durable resistance and control
 - e.g. Pyramiding quantitative and qualitative genes in new cultivars

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Phenotype data: Z. Zhong, M. Lendenmann, E. Stewart

Funding: Swiss National Science Foundation

