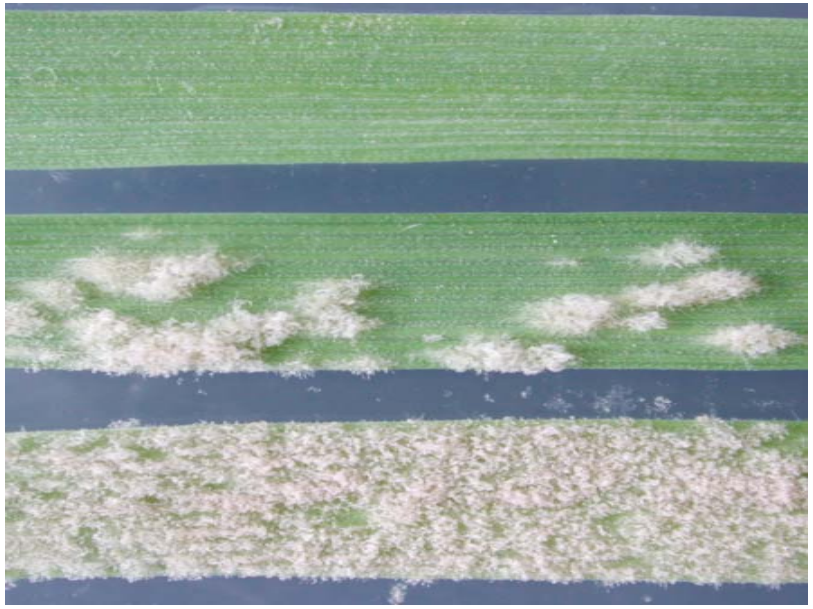
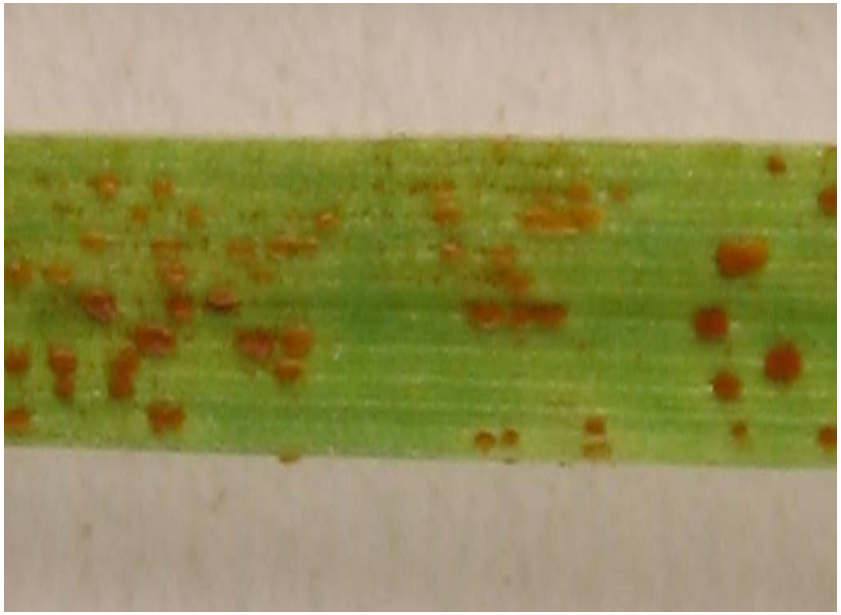




Is natural evolution providing enough diversity for wheat resistance breeding?

ITMI Workshop, PAG
January 9, 2010

Beat Keller

Universität Zürich

Cloned resistance genes in wheat

- Leaf rust resistance gene *Lr21* (Huang et al., 2003; 2009)
- Leaf rust resistance gene *Lr10* (Feuillet et al., 2003)
- Leaf rust resistance gene *Lr1* (Cloutier et al. 2007, Qiu et al. 2007)
- Powdery mildew resistance gene *Pm3* (Yahiaoui et al., 2004; 2006; Srichumpa et al. 2005)
- Stripe rust resistance *Yr36* (Fu et al. 2009)
- QTL: leaf rust resistance *Lr34* (Krattinger et al. 2009)
- Coming up: *Sr2*, *FHB...*

Enormous genetic diversity existing and available for wheat:

- **Wild and domesticated species, landraces and elite cultivars (recent evolutionary events)**
- **Different ploidy levels (2n, 4n, 6n)**
- **Large number of relatives of wheat in the plant family of the grasses: comparative genomics (long evolutionary periods)**

What can we learn on resistance gene evolution based on the large genetic resources available?

Studies on the molecular diversity in the wheat gene pool: *Lr1*, *Lr10, Lr34* and *Pm3* alleles

Where do the modern resistance genes come from?

When did the resistance activities evolve?

How did the resistance genes evolve at the molecular level?

Artificial evolution for new resistance genes?

Wheat evolution

5 to 7 MYA

e.g. Aegilops speltoides

400,000 years

BB

Triticum urartu

AA

T. monococcum

A^mA^m

10,000 years

Triticum turgidum subsp. dicoccoides,
wild, AABB

Aegilops tauschii

DD

Triticum turgidum
subsp. *durum* or *dicoccum*
cultivated, AABB

Triticum aestivum

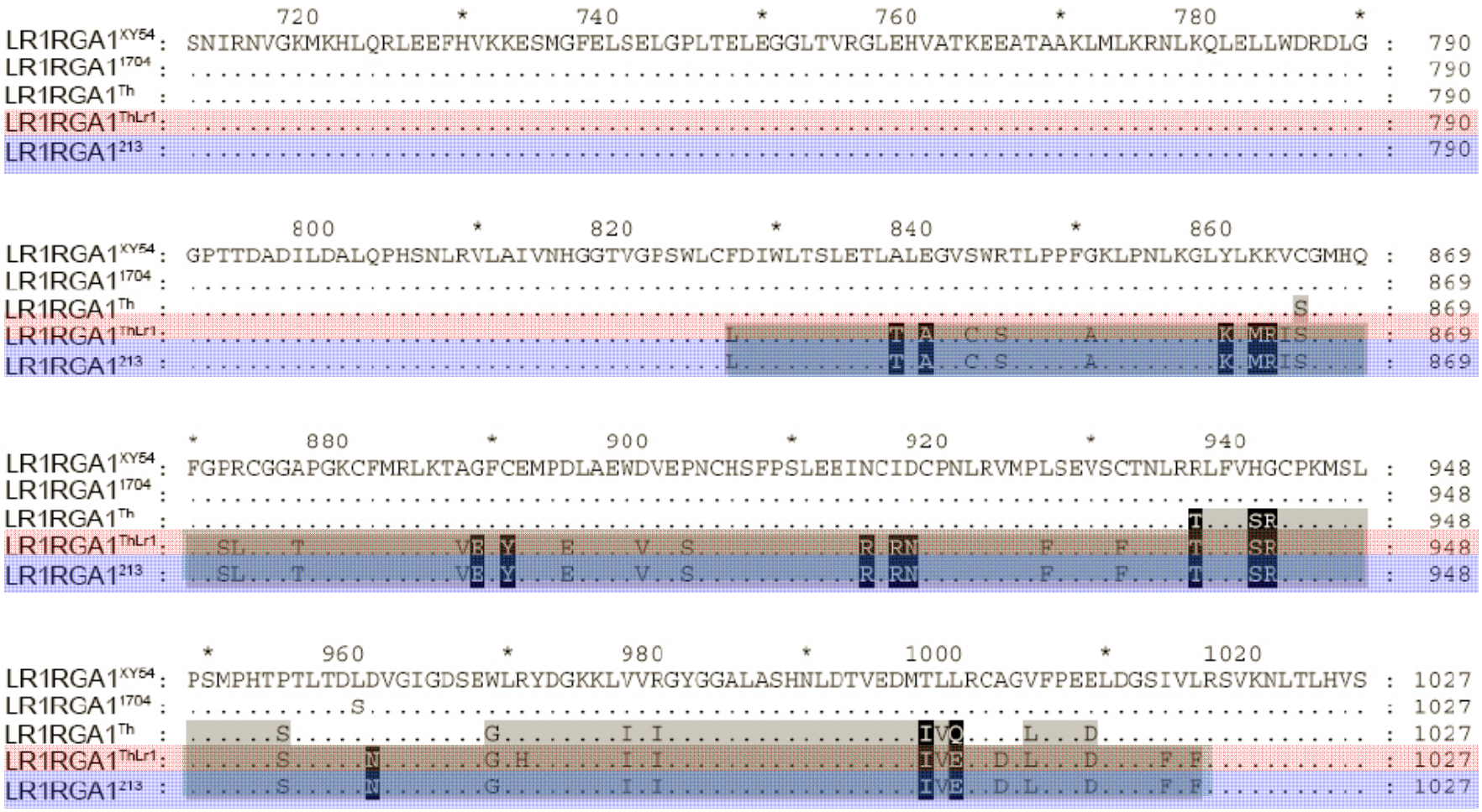
AABBDD

Origin and evolution of the *Lr1* gene: A CC-NBS-LRR coding gene

We have earlier found an *Aegilops tauschii* line (accession 213) with a leaf rust gene with identical gene location (5DL) and race-specificity as the hexaploid wheat *Lr1*.

Ling et al. 2004. Theor. Appl. Genet.

Origin and evolution of the *Lr1* gene



Cloutier et al. PMB 2007; Qiu et al. TAG 2007

Thatcher *Lr1* allele

***Aegilops tauschii Lr1* allele**

Origin and evolution of the *Lr1* gene

1. A discrete, polymorphic segment is diagnostic for the *Lr1* gene and present also in the *Ae. tauschii* gene confering resistance

2. Thus, the *Lr1* gene was already present in the gene pool of *Ae. tauschii* before formation of the hexaploid species

The *Lr10* leaf rust resistance gene from hexaploid bread wheat

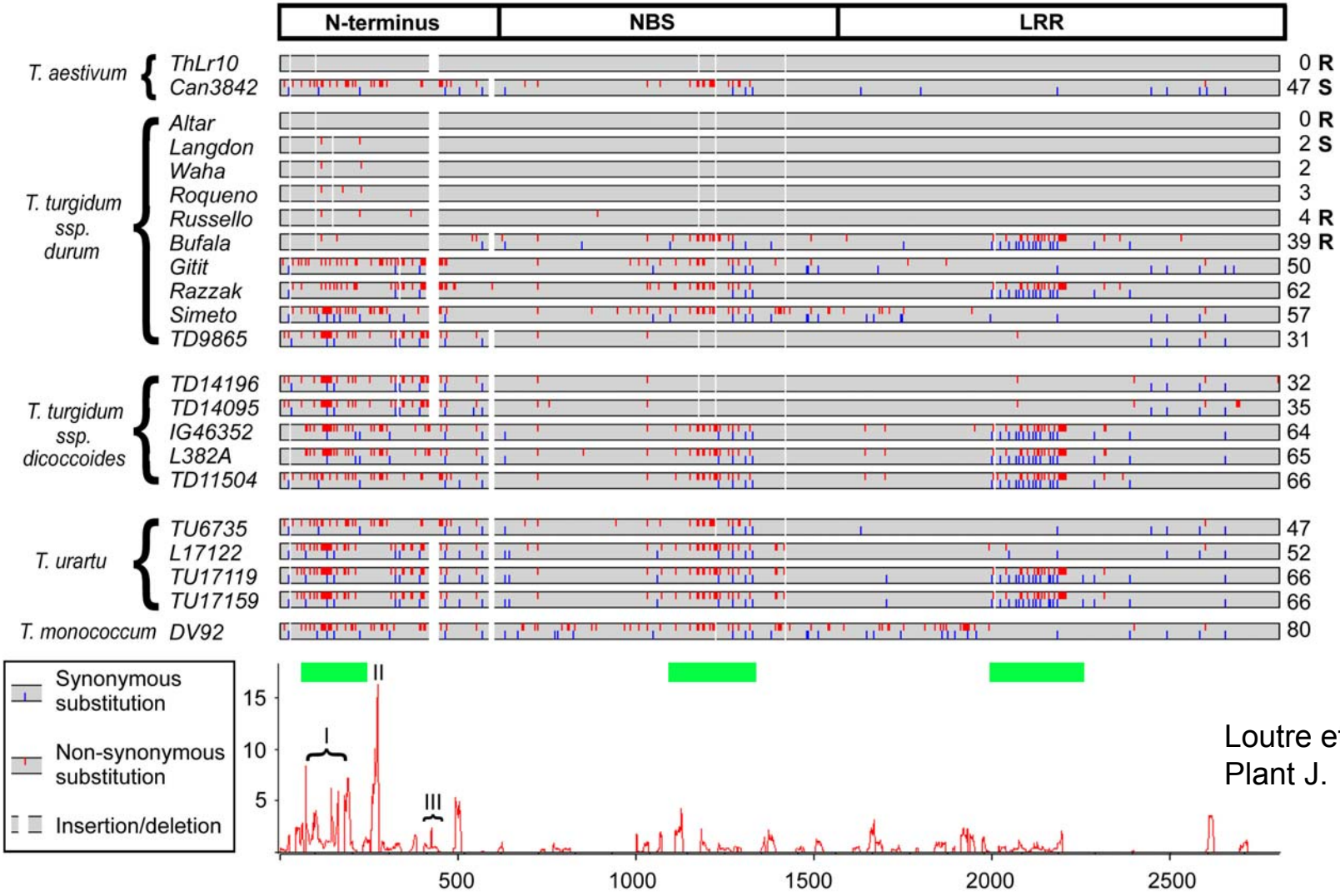
- **Widely distributed in old Australian, USA and CIMMYT varieties**
- **No known alleles**
- **May play a role in gene combinations (+*Lr13*)**

The *Lr10* leaf rust resistance gene: Diversity of *Lr10* sequences in the wheat gene pool

- **Studies of diploid, tetraploid and hexaploid wheat**

- **Isolation of *Lr10* orthologs/alleles and sequence analysis (21 sequences)**

Comparison of *Lr10* coding sequences from different wheat species



Loutre et al. 2009
Plant J.

High diversity identified in the N-terminal domain

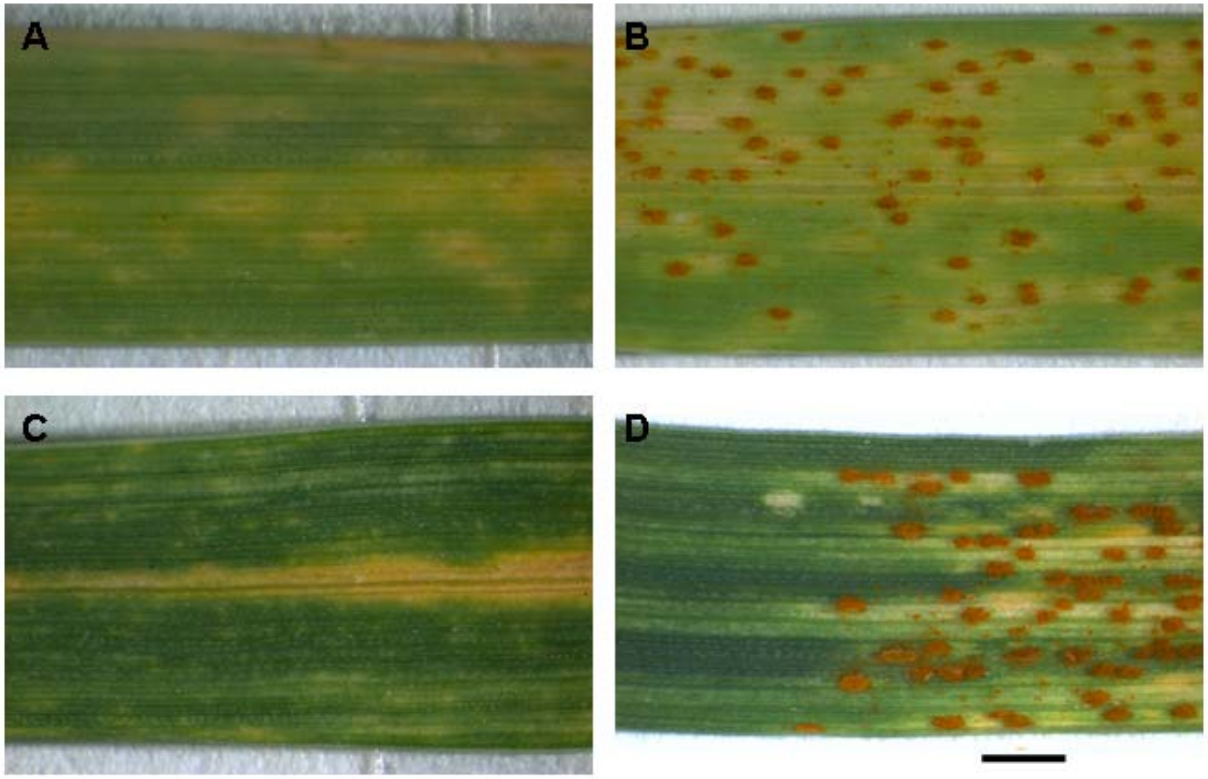
Little variation in the LRR domain with exception of polymorphic blocks

BSMV-dependent silencing of the *Lr10* gene (in hexaploid and tetraploid wheat)

Control (*Lr1*) construct **Construct targeting *Lr10***

Th*Lr10*

Tetraploid *cv.* Russello



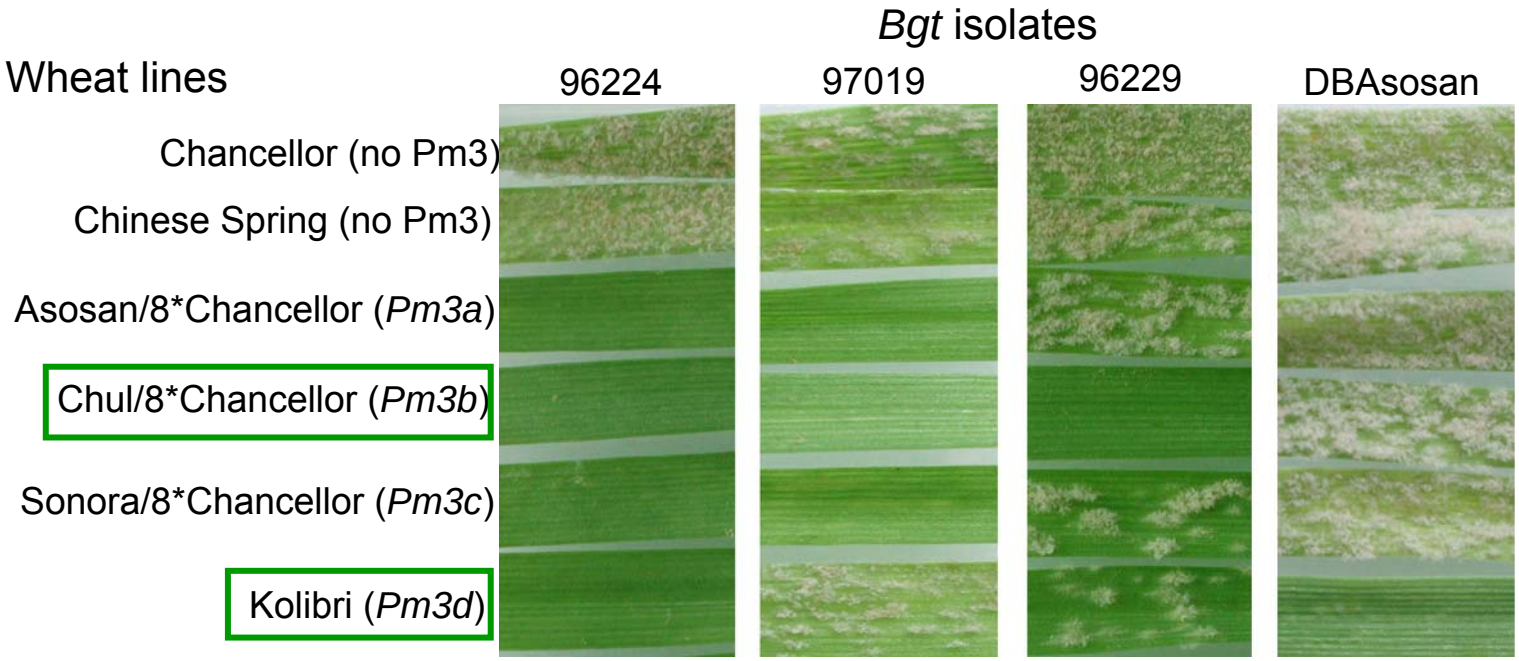
Conclusions concerning *Lr10* evolution and origin:

A gene identical to *Lr10* is found in tetraploid line „Altar“.
Therefore, the origin of *Lr10* is in the tetraploid gene pool, maybe earlier

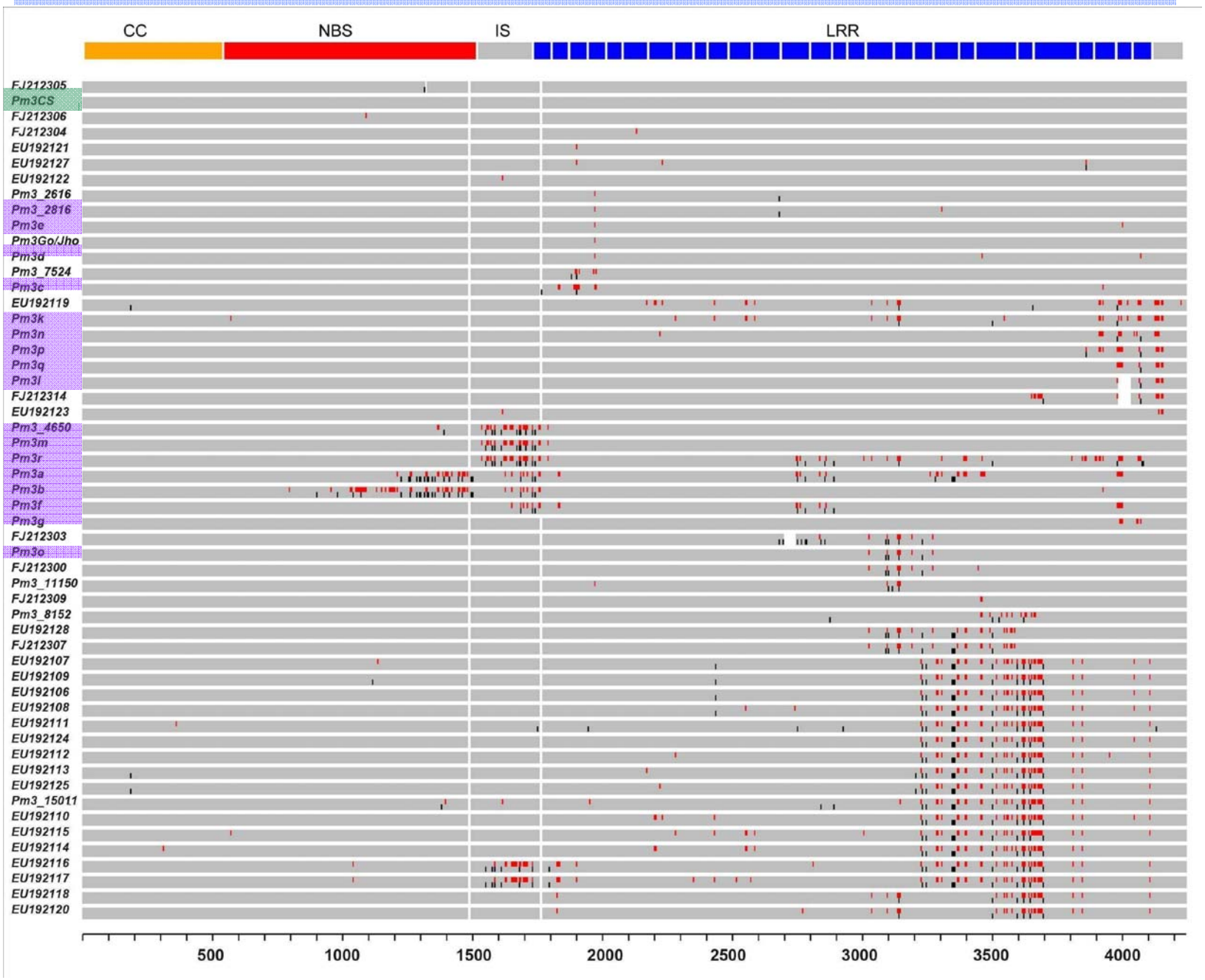
There are functional, diverse alleles of *Lr10* in the tetraploid gene pool

The *Pm3* resistance alleles

- chromosome 1AS (Briggle and Sears, 1966; McIntosh and Baker,1968)
- 17 functional alleles known (2 unpublished) (*Pm3a to s*)
- race-specific resistance gene to *Blumeria graminis* f.sp. *tritici*



The 17 *Pm3* resistance alleles are highly similar in sequence

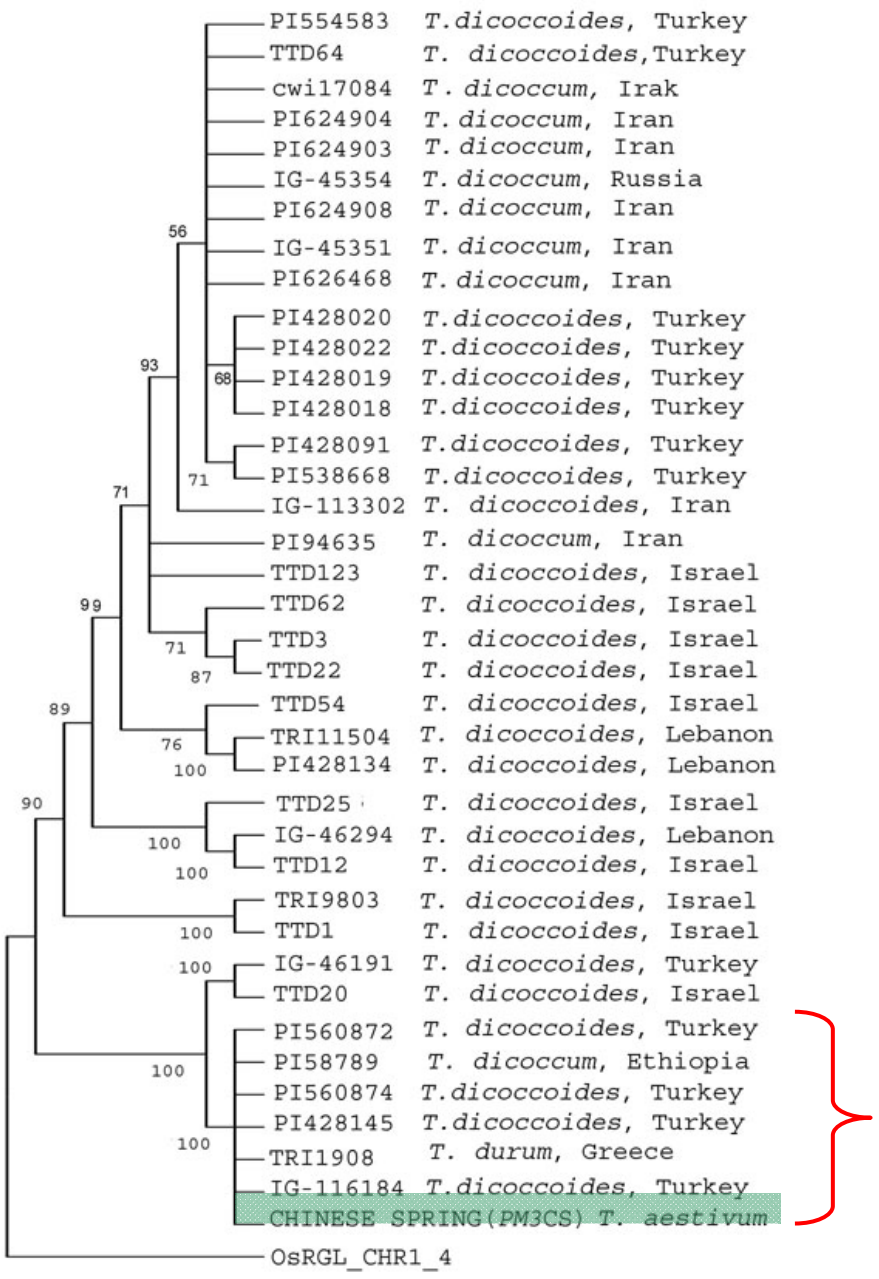


► The consensus sequence of the resistant *Pm3* alleles corresponds to a naturally occuring susceptible allele (Chinese Spring *Pm3CS* allele)

►We have found that this *Pm3CS* allele is widespread in the gene pool of hexaploid bread wheat

►Thus, the resistance alleles are derived from a closely related, susceptible template sequence

How did the functional alleles of *Pm3* evolve?

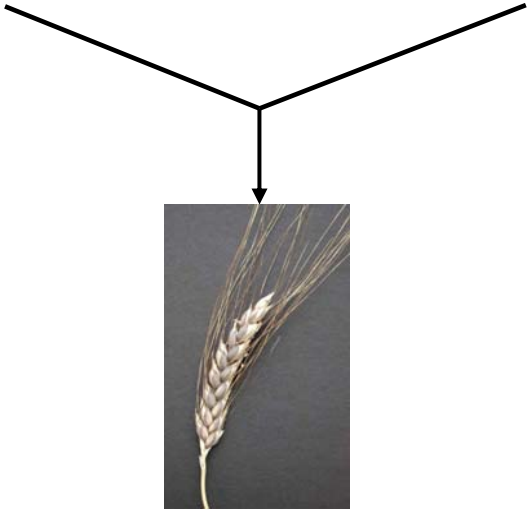


Analysis of *Pm3* gene diversity in wild (and some domesticated) tetraploid wheat

None of the functional *Pm3* genes in 6n wheat was found in 4n wheat.

There is only one common allele in tetraploid and hexaploid wheat, the susceptible *Pm3CS* consensus sequence of all resistance alleles

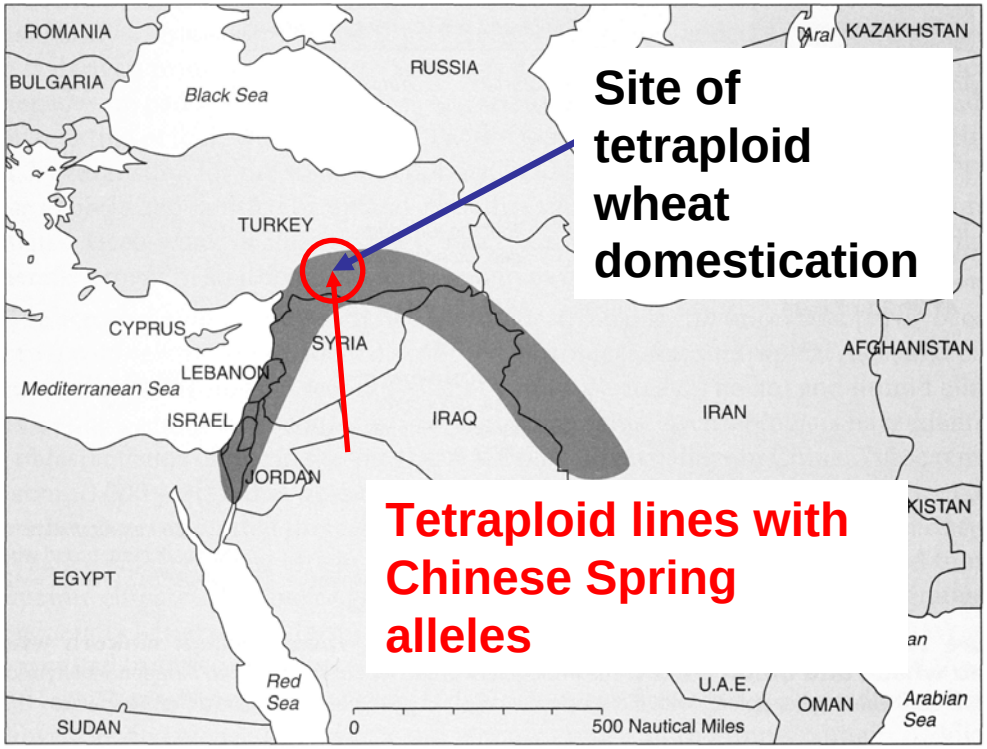
Evolution of the *Pm3* resistance gene



Triticum turgidum

AABB

wild and domesticated



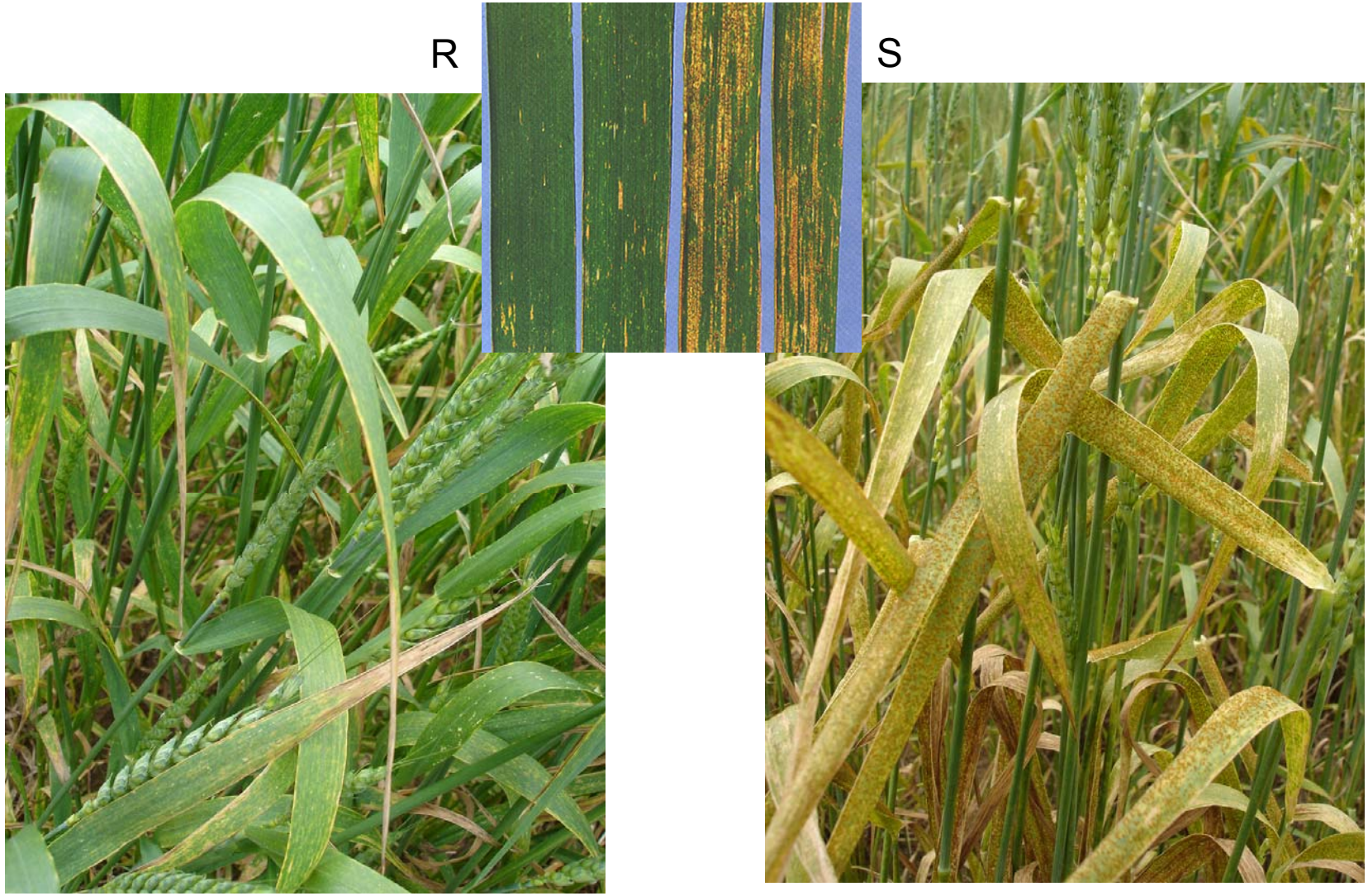
Model for *Pm3* resistance gene evolution

- A tetraploid, domesticated wheat with the modern susceptible Chinese Spring *Pm3CS* allele was involved in the hybridization events resulting in bread wheat

- New resistance genes evolved since domestication (8,000 years) based on the susceptible *Pm3CS* template gene sequence

- These new alleles were selected in agroecosystems by humans because of their positive value

Lr34: a durable leaf rust resistance gene

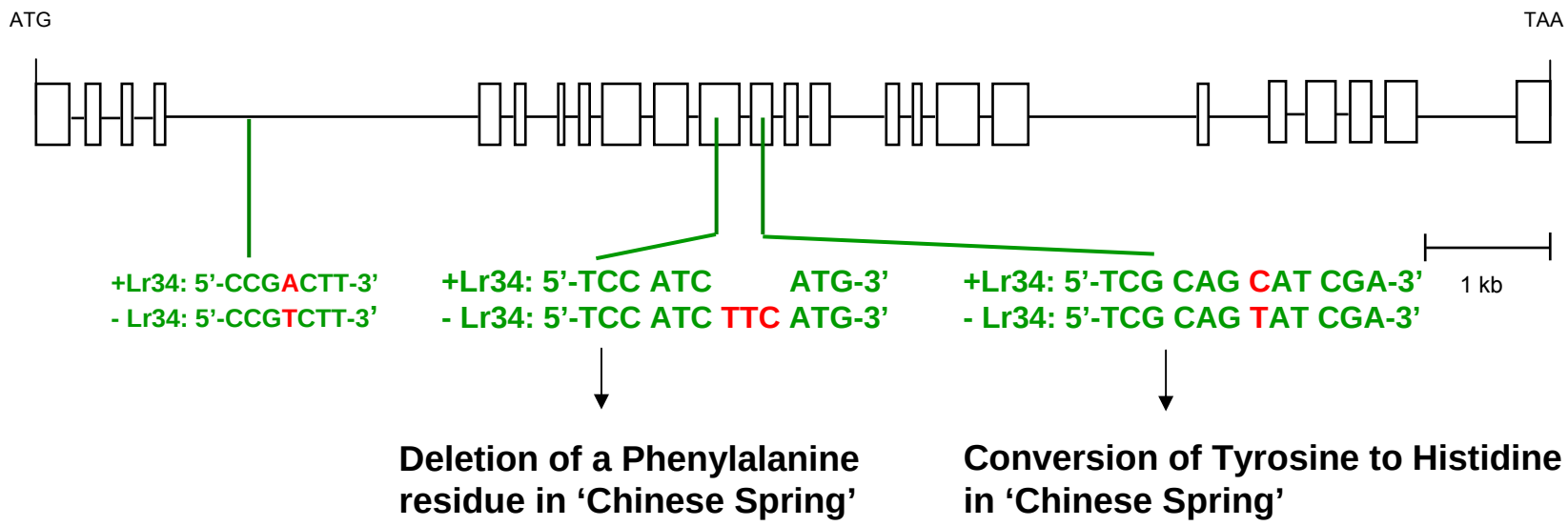


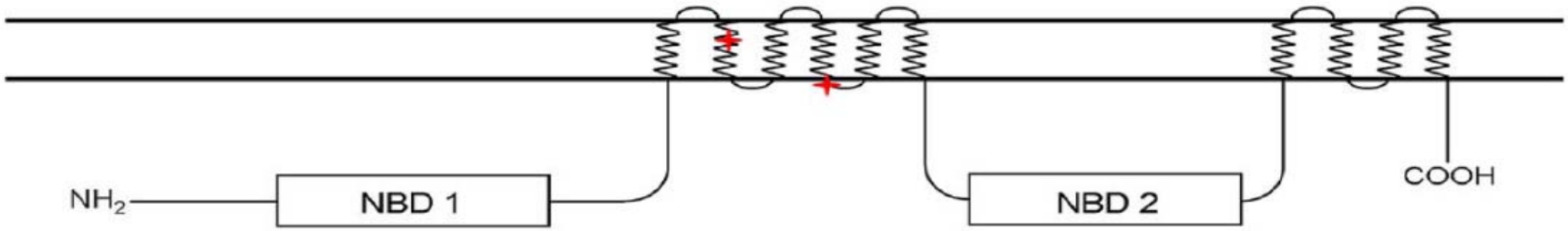
Thatcher + *Lr34*

Thatcher

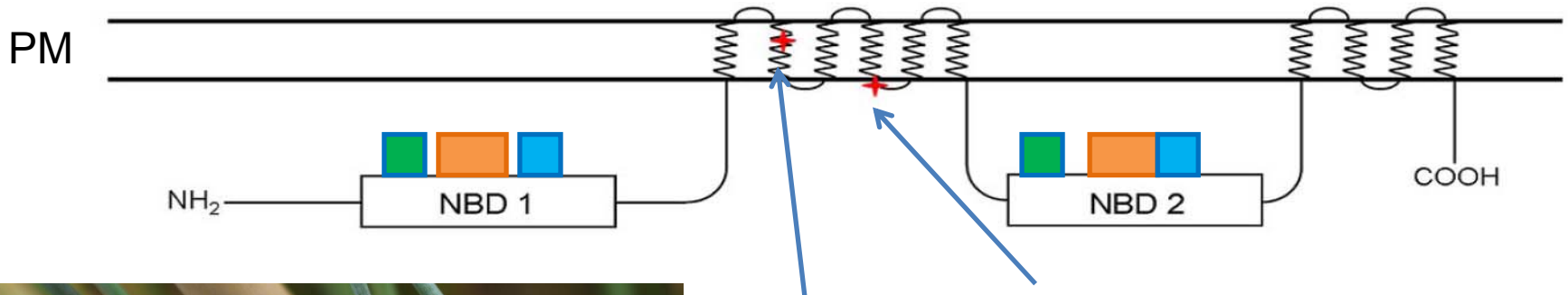
Sequence differences between + / - *Lr34* alleles

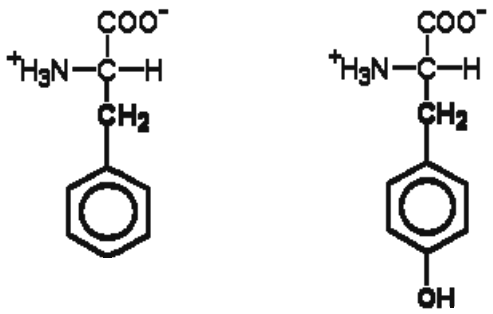
Sequence comparison of the +*Lr34* line 'Chinese Spring' and the –*Lr34* French winter wheat cultivar 'Renan'.





The ‚susceptible' allele of *Lr34*

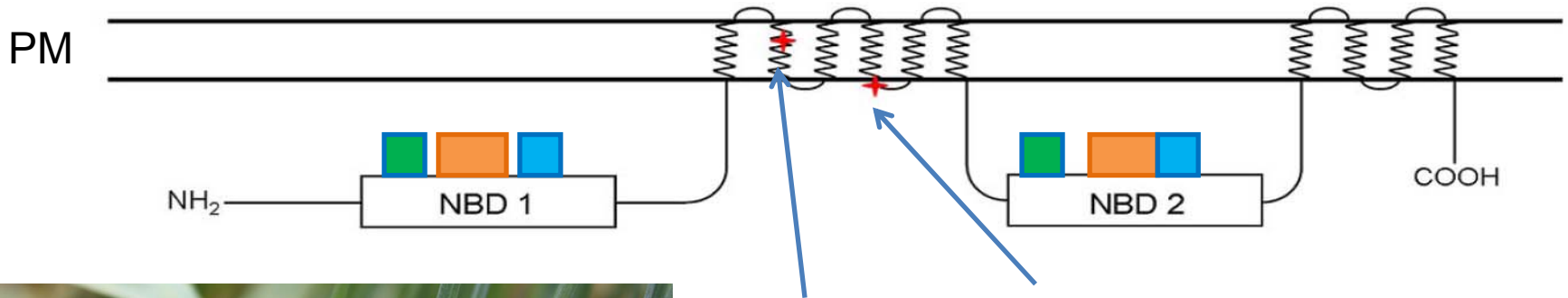


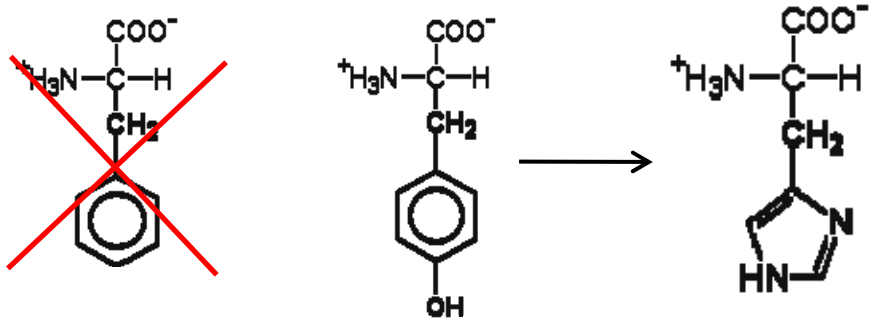


Phe: TMH2

Tyr: Cytosolic loop between TMH4&TMH5

The ‚resistant' allele of *Lr34*





Deletion of Phe **Conversion of Tyr→His**

→Resistant and susceptible allele are expressing putatively functional proteins

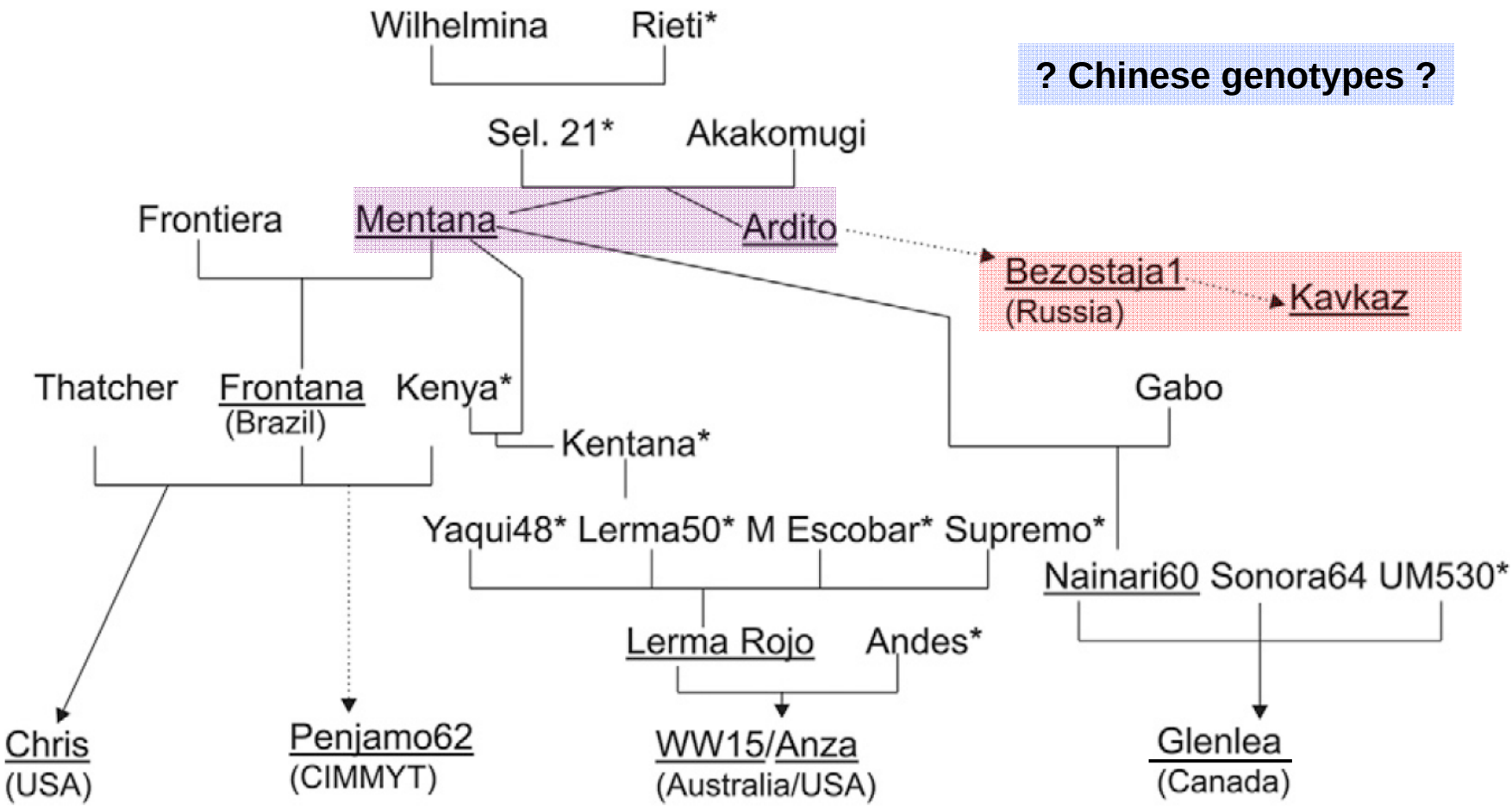
Sequence differences between + / - *Lr34* alleles

	genotype	origin	+/- Lr34	A/T SNP intron 4	3bp deletion exon 11	C/T SNP exon 12
Far East	Chinese Spring	China	+	A	Deletion	C
	RL6058	China	+	A	Deletion	C
	Fukuho	Japan	+	A	Deletion	C
Spring wheat (North / South America)	Mentana	Italy	+	A	Deletion	C
	Frontana	Brazil	+	A	Deletion	C
	Fronteira	Brazil	-	T	No deletion	T
	Ardito	Italy	+	A	Deletion	C
	Jupateco R	CIMMYT	+	A	Deletion	C
	Jupateco S	CIMMYT	-	T	No deletion	T
	Glenlea	Canada	+	A	Deletion	C
	Thatcher	Canada	-	T	No deletion	T
	Anza	USA	+	A	Deletion	C
	Chris	USA	+	A	Deletion	C
	Condor	Australia	+	A	Deletion	C
	Penjamo 62	CIMMYT	+	A	Deletion	C
	Inia 66	CIMMYT	-	T	No deletion	T
	Lalbahadur Lr34	CIMMYT	+	A	Deletion	C
	Lalbahadur	India	-	T	No deletion	T
European Winter wheat	Forno	Switzerland	+	A	Deletion	C
	Arina	Switzerland	-	T	No deletion	T
	Pegaso	Italy	+	A	Deletion	C
	Bezostaja	Russia	+	A	Deletion	C
	Kavkaz	Russia	+	A	Deletion	C
	Roazon	France	-	T	No deletion	T
	Capelle Desprez	France	-	T	No deletion	T
	Maris Huntsman	UK	-	T	No deletion	T
	Renan	France	-	T	No deletion	T

The three reported sequence polymorphisms form a conserved haplotype.

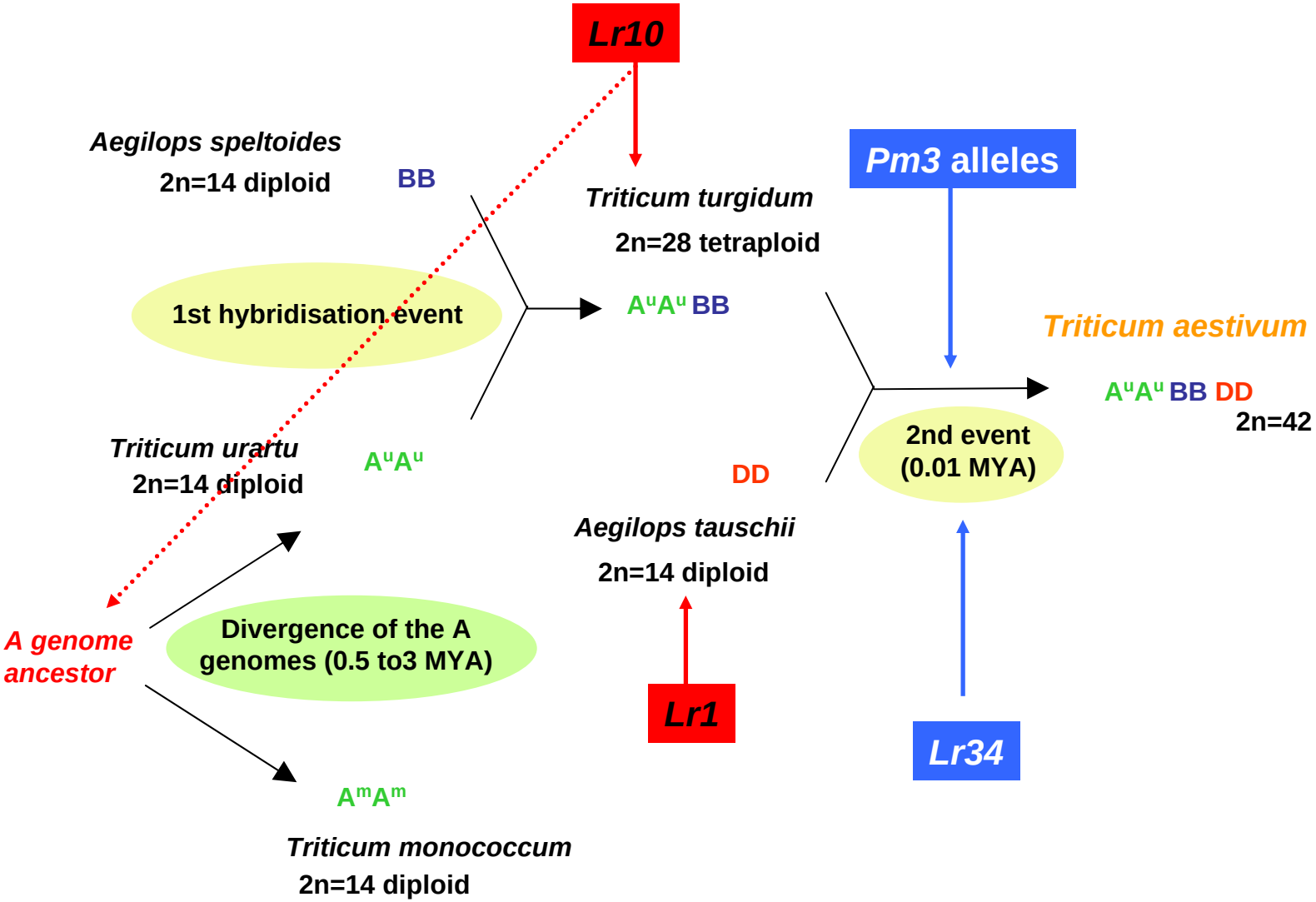
A single progenitor in an old wheat landrace likely accounts for the origin of *Lr34.*

Documented history of *Lr34* begins at the end of the 19th century in Italy and is restricted to hexaploid wheat



Kolmer et al. 2008. Crop Science. Pedigree of *Lr34/Yr18* cultivar entries in different wheat breeding programs.

Evolution of three wheat disease resistance genes: older and younger genes



Can natural evolution be complemented with artificial evolution to improve wheat resistance breeding?

Obviously, there is quite some evolution occuring since domestication

This is a very short evolutionary period

There is a good chance that many functional alleles have not yet evolved

PNAS

Artificial evolution extends the spectrum of viruses that are targeted by a disease-resistance gene from potato

Garry Farnham* and David C. Baulcombe†

The Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, United Kingdom

Edited by Diter von Wettstein, Washington State University, Pullman, WA, and approved August 17, 2006 (received for review August 3, 2006)

We lack the fast experimental systems in wheat to recognize new functional variants

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