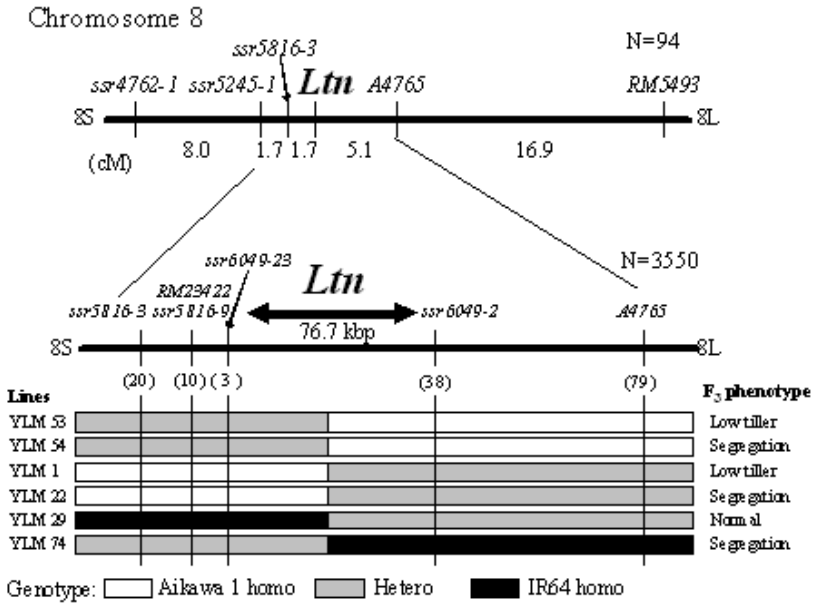
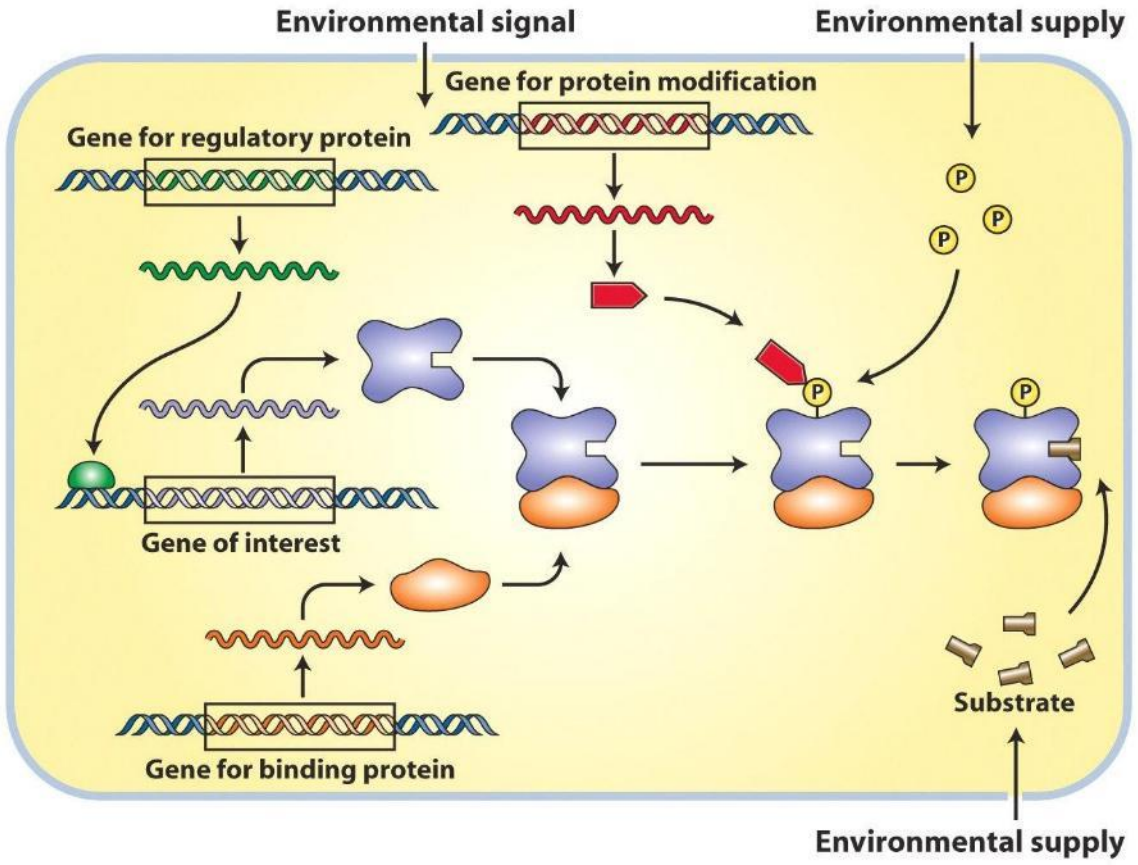
Genes to Phenotypes

"A set of genes represents the individual components of the biological system under scrutiny"

Modifications of the "3:1 F2 monohybrid ratio" and gene interactions are the rules rather than the exceptions



One gene - one polypeptide??



Allelic Variation

1. Many alleles are possible in a population, but in a diploid individual, there are only two alleles

2. Mutation is the source of new alleles

3. There are many levels of allelic variation, e.g.
   a. DNA sequence changes with no change in phenotype
   b. Large differences in phenotype due to effects at the transcriptional, translational, and/or post-translational levels
   c. Transposable element activity

How many alleles are possible?

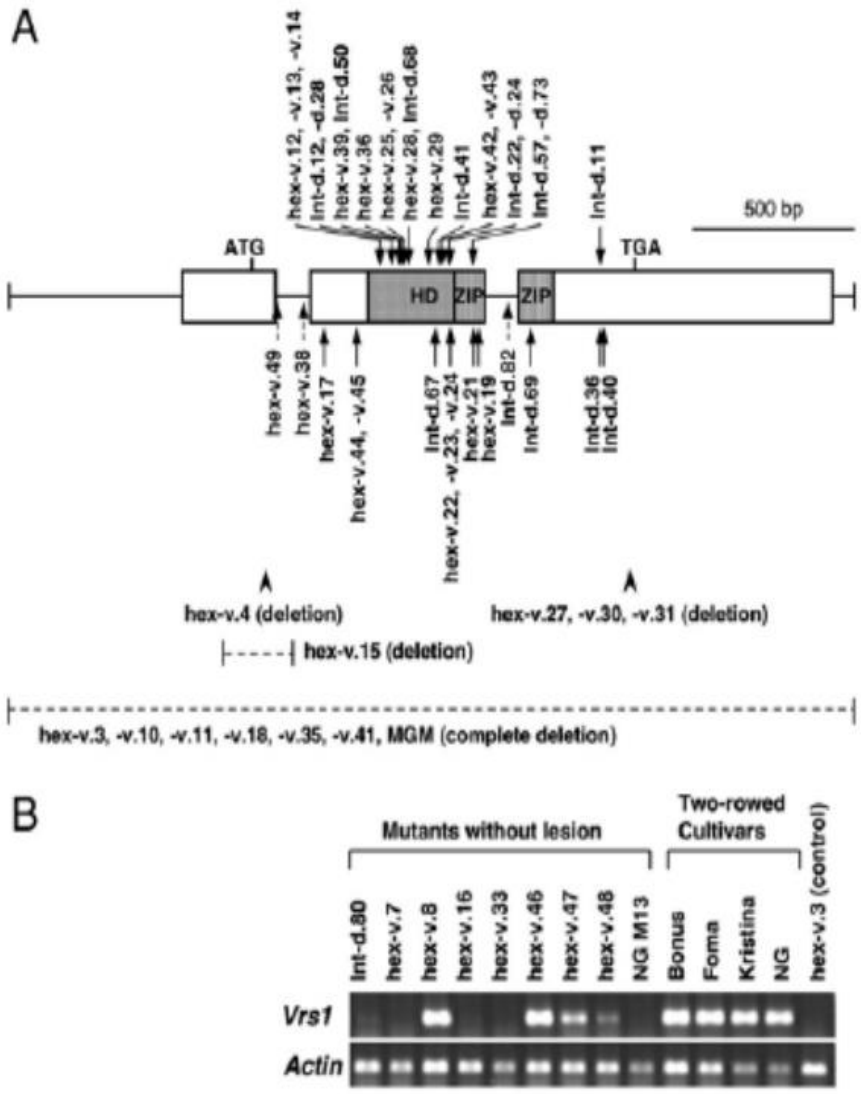


Fig. 3. Analysis of mutants allelic to *vrs1*. (*A*) Lesions at *Vrs1* detected in 48 mutants. Arrows pointing down indicate amino acid substitutions, arrows pointing up with a solid line indicate new stop codons, and the three arrows

Vrs1 Komatsuda et al. (2007) PNAS 104: 1424-1429

Allelic Relationships at a locus

Complete dominance: Deletion, altered transcription, alternative translation. The interesting case of aroma in rice: a loss of function makes rice smell great, and patent attorneys salivate....

Plant Biotechnology Journal (2005) **3**, pp. 363–370 doi: 10.1111/j.1467-7652.2005.00131.x

The gene for fragrance in rice

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accepted 22 December 2004.
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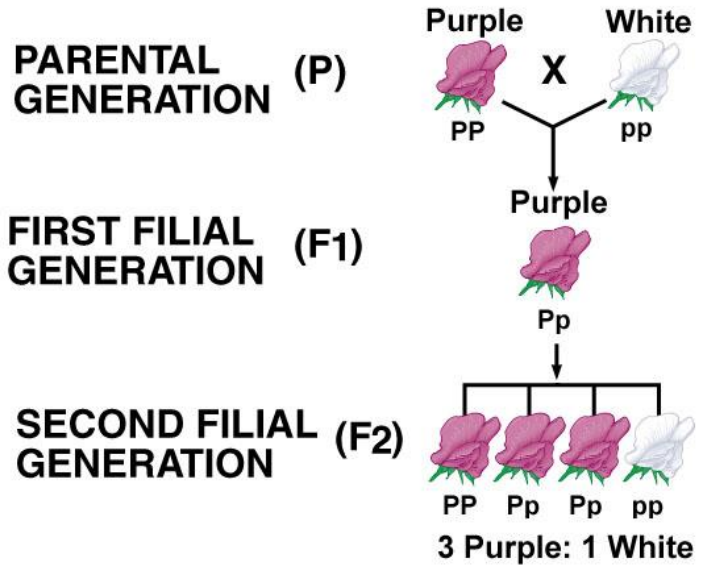
Summary

The flavour or fragrance of basmati and jasmine rice is associated with the presence of 2-acetyl-1-pyrroline. A recessive gene (*fgr*) on chromosome 8 of rice has been linked to this important trait. Here, we show that a gene with homology to the gene that encodes betaine aldehyde dehydrogenase (BAD) has significant polymorphisms in the coding region of fragrant genotypes relative to non-fragrant genotypes. The accumulation of 2-acetyl-1-pyrroline in fragrant rice genotypes may be explained by the presence of mutations resulting in a loss of function of the *fgr* gene product. The allele in fragrant genotypes has a mutation introducing a stop codon upstream of key amino acid sequences conserved in other BADs. The *fgr* gene corresponds to the gene encoding BAD2 in rice, while BAD1 is encoded by a gene on chromosome 4. BAD has been linked to stress tolerance in plants. However, the apparent loss of function of BAD2 does not seem to limit the growth of fragrant rice genotypes. Fragrance in domesticated rice has apparently originated from a common ancestor and may have evolved in a genetically isolated population, or may be the outcome of a separate domestication event. This is an example of effective human selection for a recessive trait during domestication.

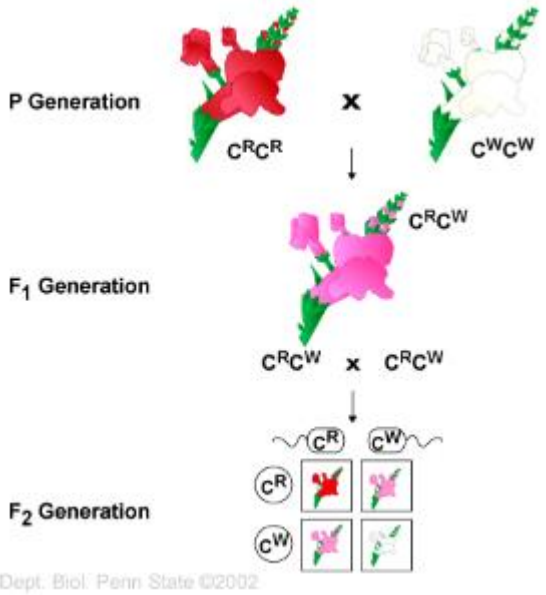
Keywords: 2-acetyl-1-pyrroline, betaine aldehyde dehydrogenase, aroma, basmati, jasmine.



Allelic Relationships at a locus

Incomplete (partial) dominance

Example: Red x White gives a pink F1. The F2 phenotypes are 1 Red: 2 Pink: 1 White.



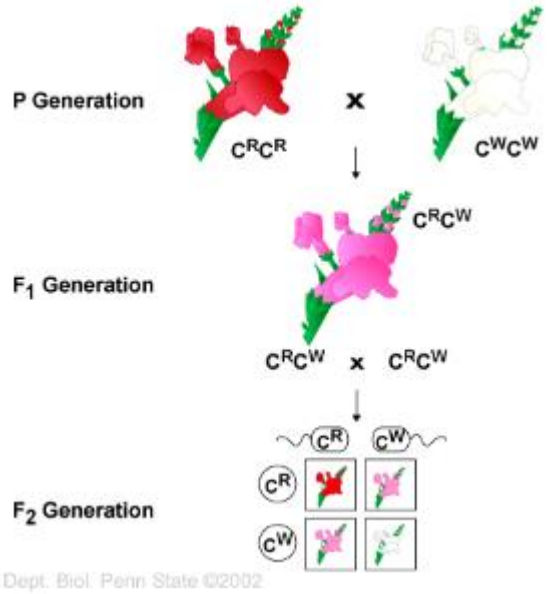
Explanation: Red pigment is formed by a complex series of enzymatic reactions. Plants with the dominant allele at the *I* locus produce an enzyme critical for pigment formation. Individuals that are *ii* produce an inactive enzyme and thus no pigment. In this case, *II* individuals produce twice as much pigment as *Ii* individuals and *ii* individuals produce none. The amount of pigment produced determines the intensity of flower color.

Allelic Relationships at a locus

Incomplete (partial) dominance

Example: Red x White gives a pink F1. The F2 phenotypes are 1 Red: 2 Pink: 1 White.

Perspectives: Enzymes are catalytic and heterozygotes usually produce enough enzyme to give normal phenotypes. This is the basis for complete dominance. However, upon closer examination, there are often measurable differences between homozygous dominant and heterozygous individuals. Thus, the level of dominance applies only to a specified phenotype.



Allelic Relationships at a locus

Codominance

An application of electrophoresis is to separate proteins or DNA extracted from tissues or whole organisms. An electric charge is run through the supporting media (gel) in which extracts, containing proteins or DNA for separation, are placed. Proteins or DNA fragments are allowed to migrate across the gel for a specified time and then stained with specific chemicals or visualized via isotope or fluorescent tags. Banding patterns are then interpreted with reference to appropriate standards. The mobility of the protein or DNA is a function of size, charge and shape.

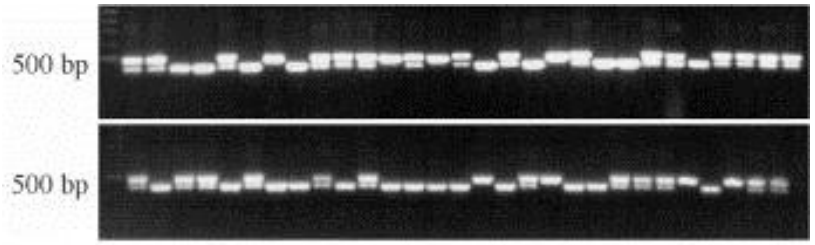


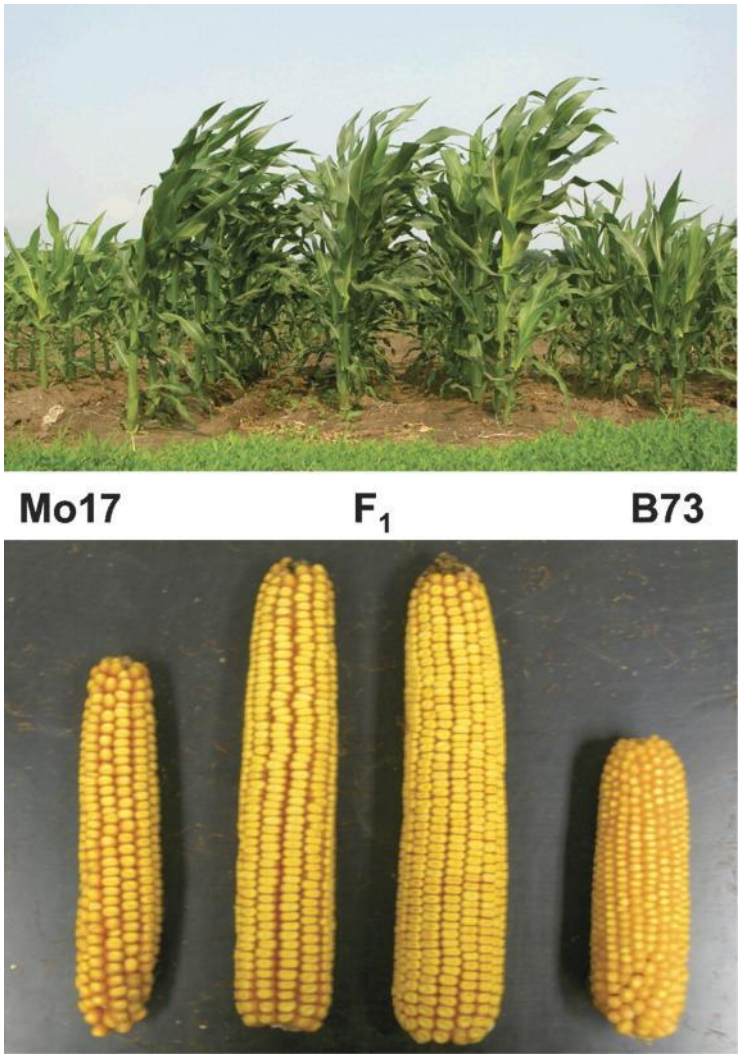
Figure 3 - The co-dominant SCAR GGTCTT-4/H separated on a 2% agarose gel, where first left lanes are size markers. Progenies from a B493 x QAL F_2 population (top) and from a Brasilia x HCM F_2 population (bottom).

Allelic Relationships at a locus

Overdominance

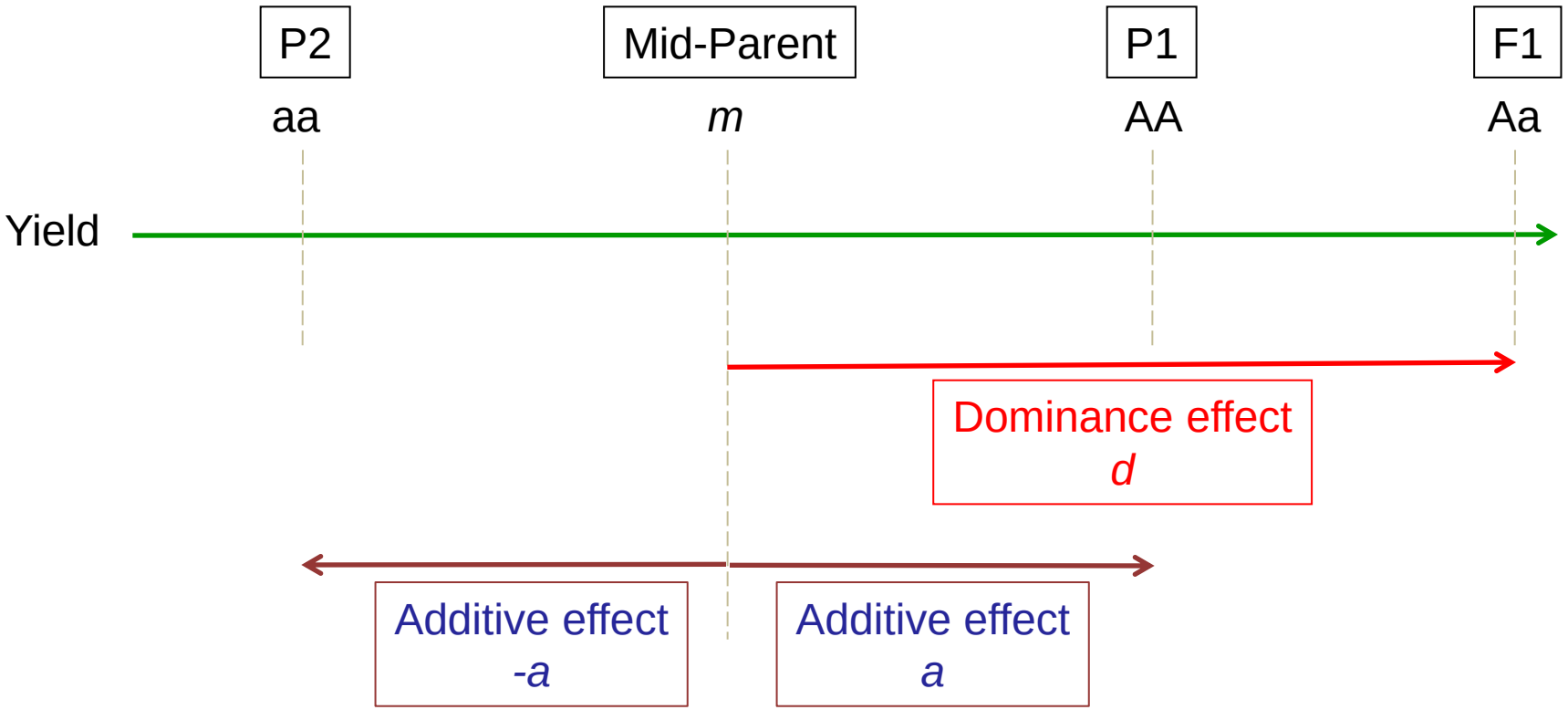
Cross two lines together and the F1 deviates significantly from the mid-parent

Segregation and independent assortment in F2



Hybrid Vigor (Heterosis)

Single Gene Model



Heterosis

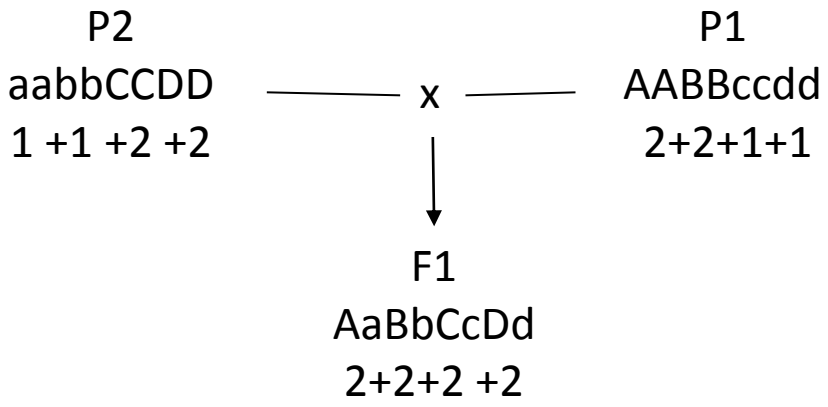
- Significantly exceed mid-parent
  - F1 > (P1+P2)/2; AA>Aa>(0.5*(AA+aa))>aa
- Significantly exceed best parent
  - F1 > P1; Aa>AA>aa
    - Most commercially useful

Cause of Heterosis

- Over-dominance theory
  - Heterozygous advantage, $d > a$
  - F1's always better than inbreds
- Dispersed dominant genes theory
  - Character controlled by a number of genes
  - Favourable alleles dispersed amongst parents ($d \leq a$)
  - Can develop inbreds as good as F1

Dispersed Dominance

- Completely dominant genes shared by parents
  - Maximum heterosis when parents are fixed for opposite alleles and dominance is complete

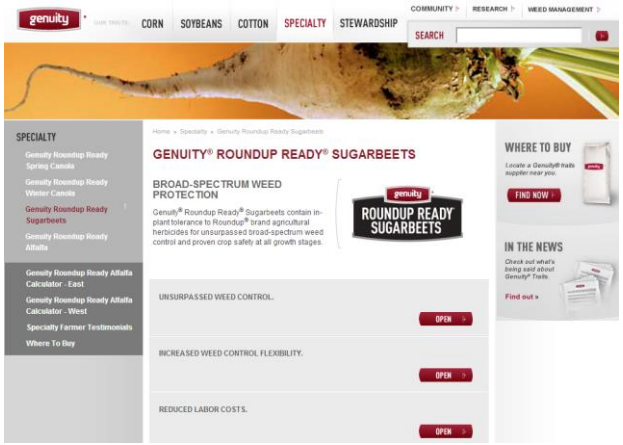


The molecular basis of heterosis

Schnable, P., and N. Springer. 2013. Progress toward understanding heterosis in crop plants. Annu. Rev. Plant Biol.64:71-88

Involves structural variation:

- SNPs and INDELs
- SV (structural variation)
- CV (copy number variation)
- PAV (presence/absence variation)



Involves differences in expression level:

- The majority of genes differentially expressed between parents expressed at mid-parent level In the F1
- Some non-additive expression

Involves epigenetics

The molecular basis of heterosis

Schnable, P., and N. Springer. 2013. Progress toward understanding heterosis in crop plants. Ann. Rev. Plant Biol.64:71-88

Conclusions:

1. No simple, unifying explanation for heterosis
    - Species, cross, trait specificity

2. Extensive functional intra-specific variation for genome content and expression

3. Heterosis generally the result of the action of multiple loci: quantitative inheritance

Non-Allelic Interactions

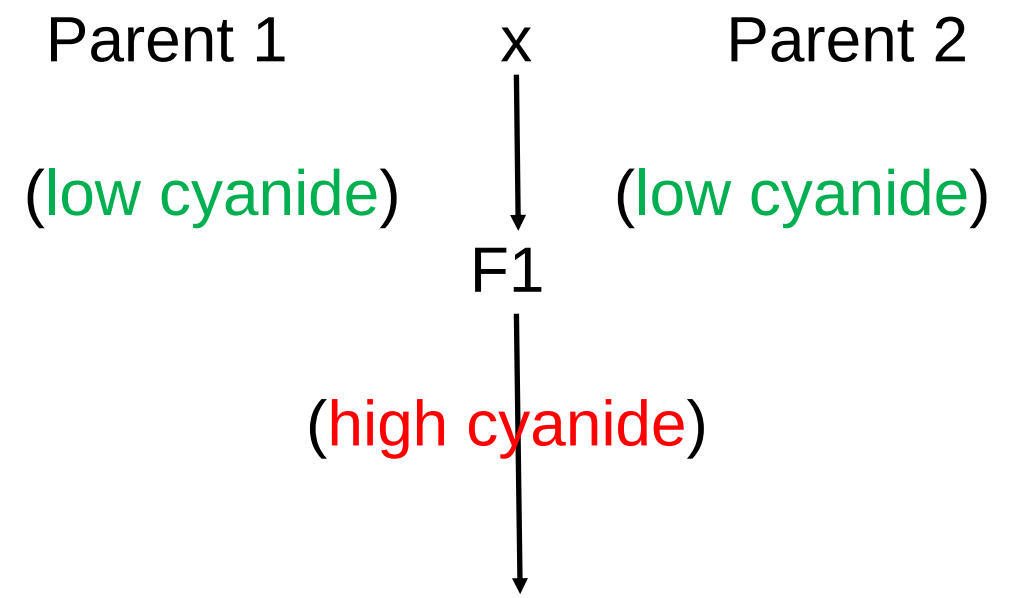
Epistasis: Interaction between alleles at different loci

Example: Duplicate recessive epistasis
(Cyanide production in white clover).
Identical phenotypes are produced when either locus is homozygous recessive (A-bb; aaB-), or when both loci are homozygous recessive (aabb).

Duplicate Recessive Epistasis

Cyanide Production in white clover

Parental, F1, and F2 phenotypes:



Duplicate Recessive Epistasis

AAbb *Low Cyanide* x aaBB *Low Cyanide*

F1 AaBb *High Cyanide*

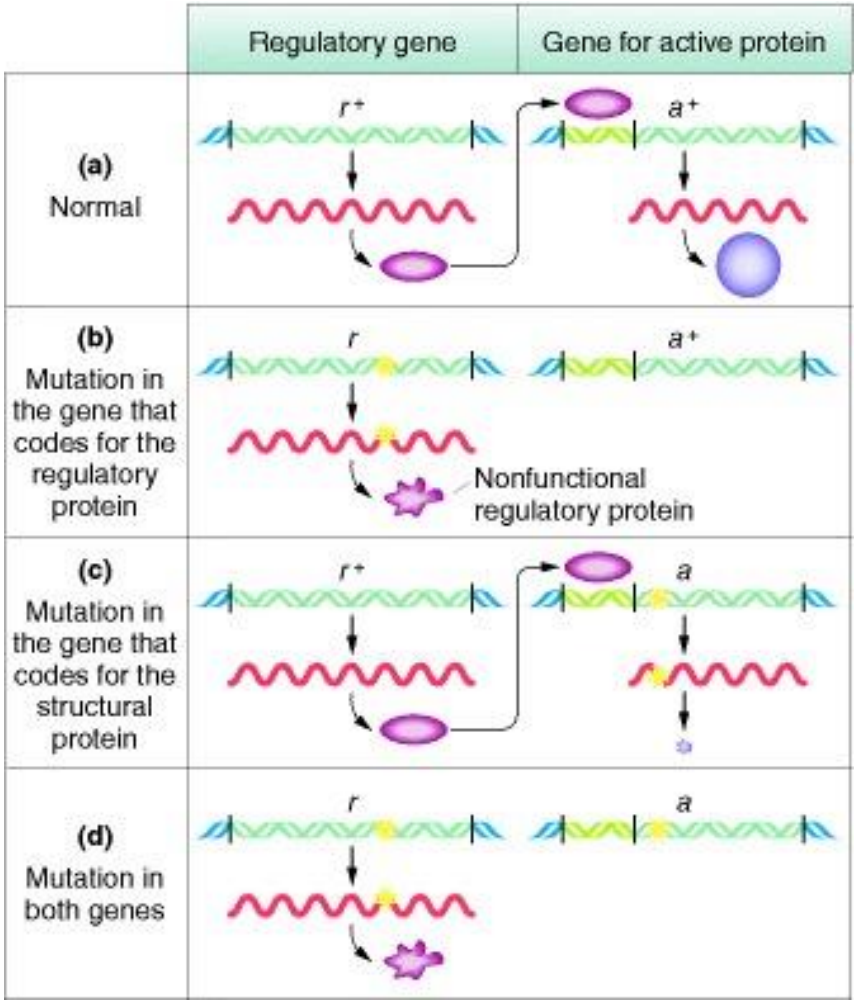
F2

	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

9 High : *7 Low Cyanide*

Doubled Haploid Ratio??

Duplicate Recessive Epistasis



Precursor → *Enzyme 1* (AA; Aa) → **Glucoside** → *Enzyme 2* (BB; Bb) → **Cyanide**

If Enzyme 1 = aa; end pathway and accumulate Precursor; if Enzyme 2 = bb; end pathway and accumulate Glucoside

Dominant Epistasis

Example: Fruit color in summer squash (*Cucurbita pepo*)

Plant 1 has white fruit and Plant 2 has yellow fruit; the F1 of a cross between them has yellow fruit

x

Selfing the F1 gives a ratio of 12 white, 3 yellow and 1 green fruited plants

Dominant Epistasis

Example: Fruit colour in summer squash (*Cucurbita pepo*)

WWyy *White Fruit* x wwYY *Yellow Fruit*

↓

F1 AaBb *White Fruit*

F2

	WY	Wy	wY	wy
WY	WWYY	WWYy	WwYY	WwYy
Wy	WWYy	Wwyy	WwYy	Wwyy
wY	WwYY	WwYy	wwYY	wwYy
wy	WwYy	Wwyy	wwYy	wwyy

A Dominant allele at the W locus suppresses the expression of any allele at the Y locus

W is epistatic to Y or y to give a 12:3:1 ratio

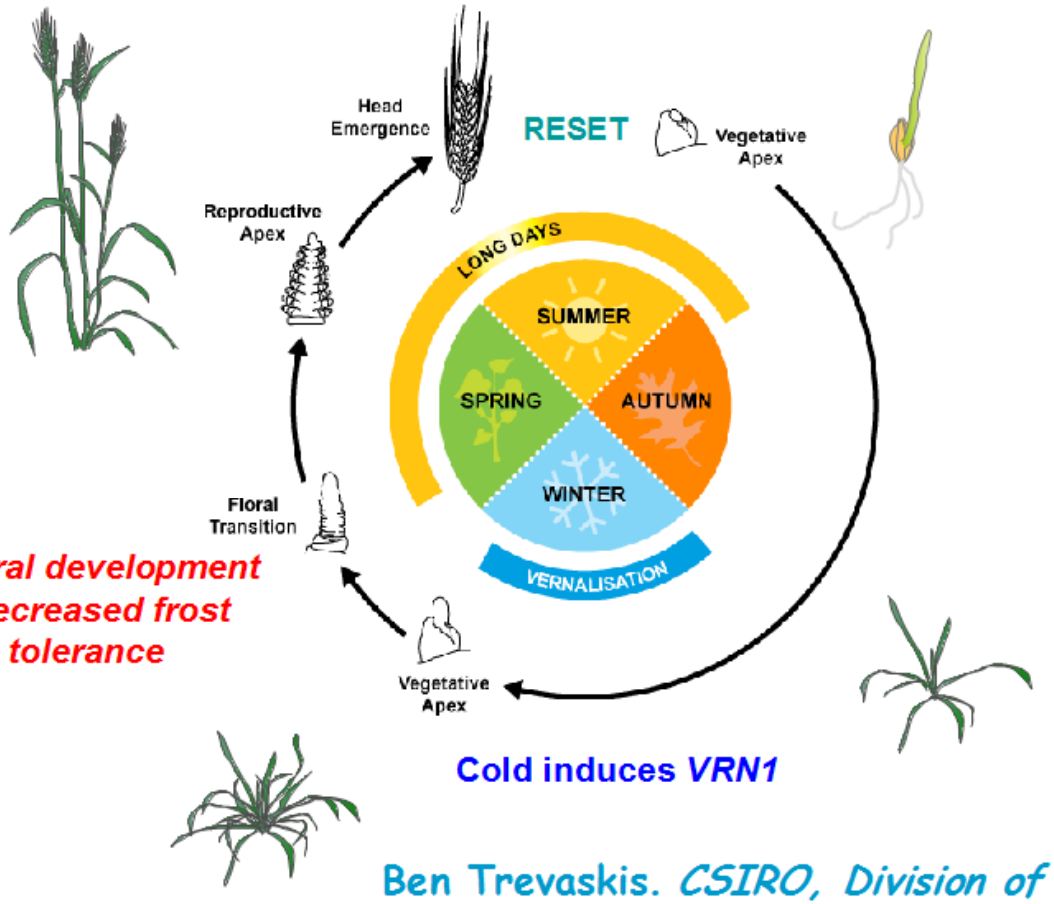
Dihybrid F2 ratios with and without epistasis

Gene Interaction	Control Pattern	A-B-	A-bb	aaB-	aabb	Ratio
Additive	No interaction between loci	9	3	3	1	9:3:3:1
Duplicate Recessive	Dominant allele from each locus required	9	3	3	1	9:7
Duplicate	Dominant allele from each locus needed	9	3	3	1	9:6:1
Recessive	Homozygous recessive at one locus masks second	9	3	3	1	9:3:4
Dominant	Dominant allele at one locus masks other	9	3	3	1	12:3:1
Dominant Suppression	Homozygous recessive allele at dominant suppressor locus needed	9	3	3	1	13:3
Duplicate Dominant	Dominant allele at either of two loci needed	9	3	3	1	15:1

Dihybrid doubled haploid ratios with and without epistasis

Gene Interaction	Control Pattern	AABB	AAbb	aaBB	aabb	Ratio
Additive	No interaction between loci	1	1	1	1	1:1:1:1
Duplicate Recessive	Dominant allele from each locus required	1	1	1	1	1:3
Duplicate	Dominant allele from each locus needed	1	1	1	1	1:2:1
Recessive	Homozygous recessive at one locus masks second	1	1	1	1	1:1:2
Dominant	Dominant allele at one locus masks other	1	1	1	1	2:1:1
Dominant Suppression	Homozygous recessive allele at dominant suppressor locus needed	1	1	1	1	3:1
Duplicate Dominant	Dominant allele at either of two loci needed	1	1	1	1	3:1

Vernalization sensitivity in cereals
Epistasis
(and epigenetics)



Ben Trevaskis. *CSIRO, Division of Plant Industry*

The phenotype: Vernalization requirement/sensitivity

- Exposure to low temperatures necessary for a timely transition from the vegetative to the reproductive growth stage

Why of interest?

- Flowering biology = productivity
- Correlated with low temperature tolerance
  - Low temperature tolerance require for winter survival
  - Many regions have winter precipitation patterns
  - Fall-planted, low temperature-tolerant cereal crops:
    - a tool for dealing with the effects climate change

The genotype: Vernalization requirement/sensitivity

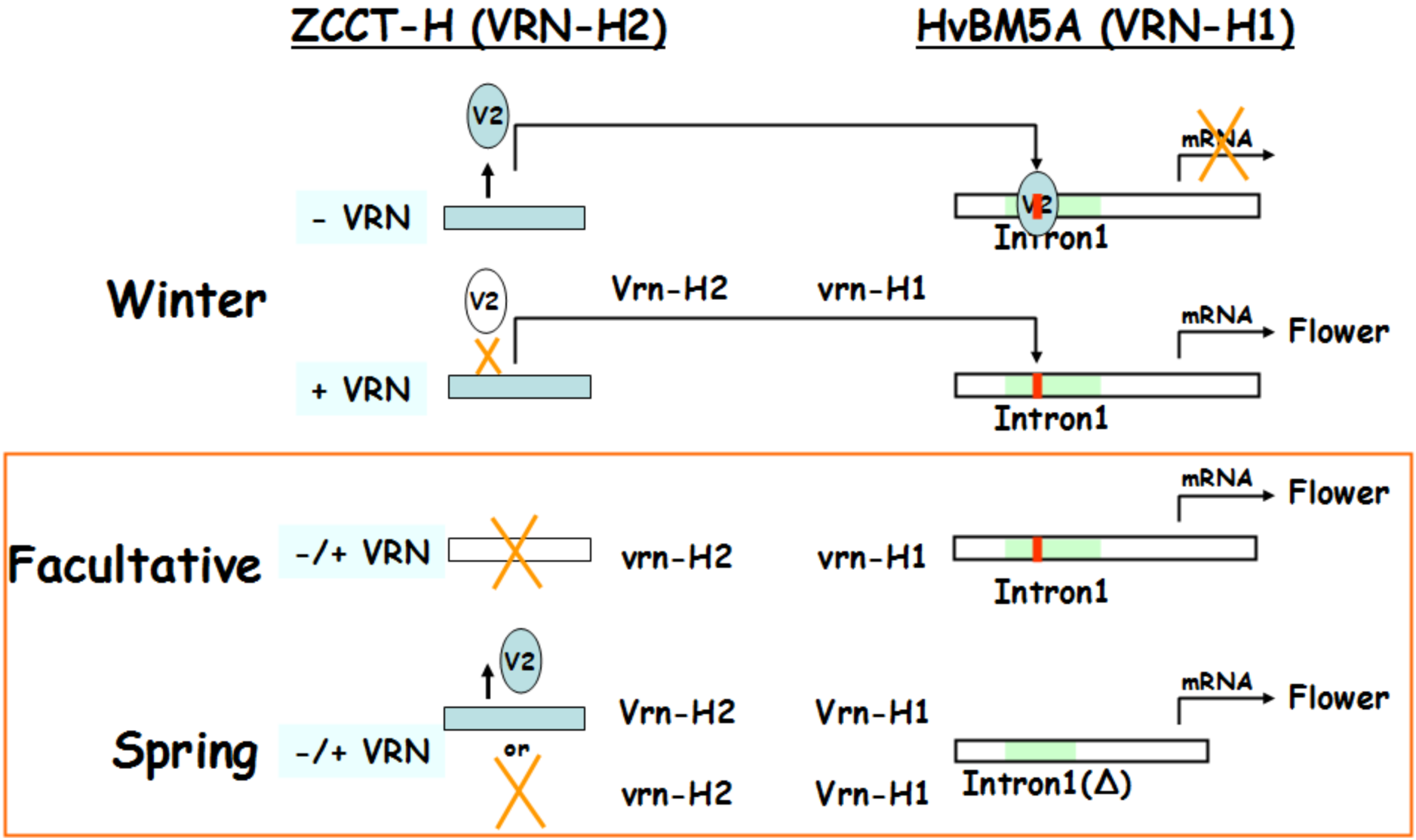
- Three locus epistatic interaction: *VRN-H1, VRN-H2, VRN-H3*

*VRN-H_* loci and allelic configurations			Vernalization sensitivity
V1	***V2***	***V3***	
V	V	V	No
V	V	*v*	No
V	*v*	V	No
V	*v*	*v*	No
v	V	V	No
v	V	*v*	Yes
v	*v*	V	No
v	*v*	*v*	No

7:1 ratio (DH)

Takahashi and Yasuda (1971)

A model for intra-locus repression and expression



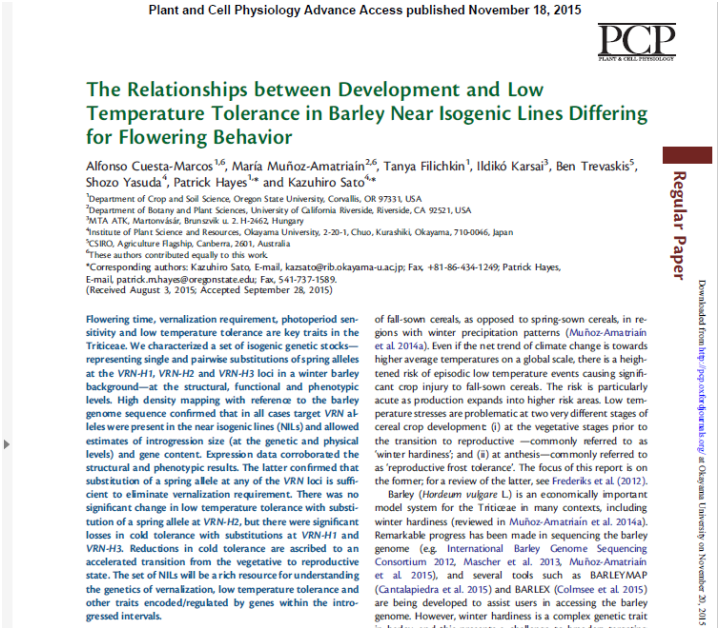
Genetics of vernalization sensitivity

- Alternative functional alleles (intron 1): *VRN-H1*
- Chromatin remodeling: *VRN-H1*
- Gene deletion: *VRN-H2*
- Copy number variation: *VRN-H3*

Understanding what Takahashi and Yasuda created, and genetic dissection of the relationships between vernalization sensitivity and low temperature tolerance

Cuesta-Marcos et al. (2015)

- SNP genotypes of parents and each isogenic line - in linkage map order
- The barley genome sequence
- Gene expression
- Low temperature tolerance and vernalization sensitivity phenotypic data



Plant and Cell Physiology Advance Access published November 18, 2015

PCP

PLANT & CELL PHYSIOLOGY

Regular Paper

The Relationships between Development and Low Temperature Tolerance in Barley Near Isogenic Lines Differing for Flowering Behavior

Alfonso Cuesta-Marcos[1,6], María Muñoz-Amatriain[2,6], Tanya Filichkin[1], Ildikó Karsai[3], Ben Trevaskis[5], Shozo Yasuda[4], Patrick Hayes[1,*] and Kazuhiro Sato[4,*]

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(Received August 3, 2015; Accepted September 28, 2015)

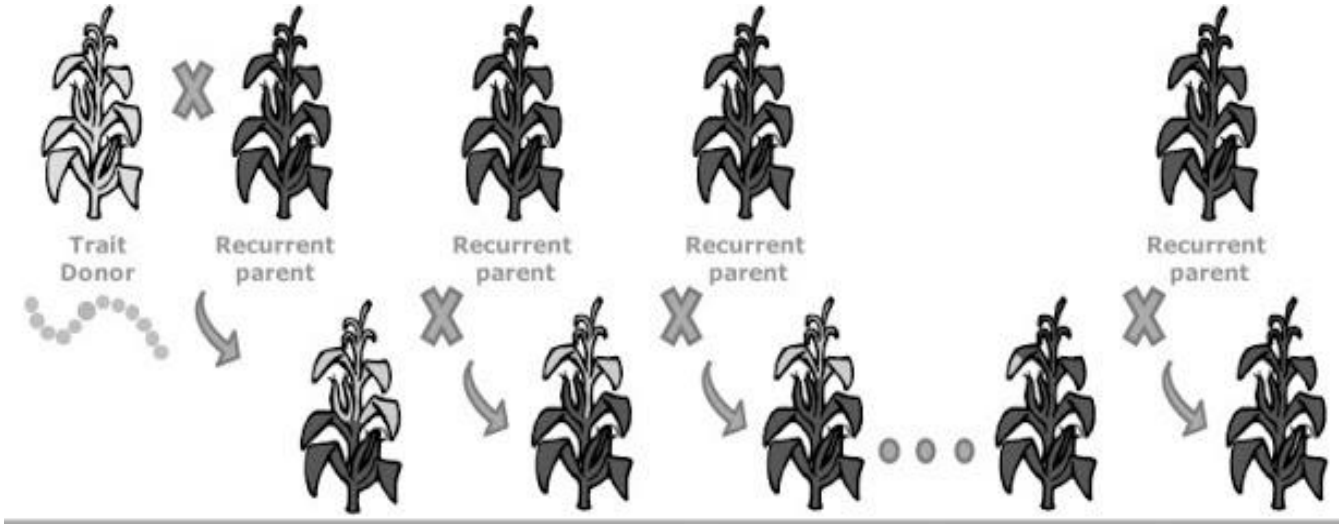
Flowering time, vernalization requirement, photoperiod sensitivity and low temperature tolerance are key traits in the Triticeae. We characterized a set of isogenic genetic stocks—representing single and pairwise substitutions of spring alleles at the *VRN-H1*, *VRN-H2* and *VRN-H3* loci in a winter barley background—at the structural, functional and phenotypic levels. High density mapping with reference to the barley genome sequence confirmed that in all cases target *VRN* alleles were present in the near isogenic lines (NILs) and allowed estimates of introgression size (at the genetic and physical levels) and gene content. Expression data corroborated the structural and phenotypic results. The latter confirmed that substitution of a spring allele at any of the *VRN* loci is sufficient to eliminate vernalization requirement. There was no significant change in low temperature tolerance with substitution of a spring allele at *VRN-H2*, but there were significant losses in cold tolerance with substitutions at *VRN-H1* and *VRN-H3*. Reductions in cold tolerance are ascribed to an accelerated transition from the vegetative to reproductive state. The set of NILs will be a rich resource for understanding the genetics of vernalization, low temperature tolerance and other traits encoded/regulated by genes within the introgressed intervals.

of fall-sown cereals, as opposed to spring-sown cereals, in regions with winter precipitation patterns (Muñoz-Amatriain et al. 2014a). Even if the net trend of climate change is towards higher average temperatures on a global scale, there is a heightened risk of episodic low temperature events causing significant crop injury to fall-sown cereals. The risk is particularly acute as production expands into higher risk areas. Low temperature stresses are problematic at two very different stages of cereal crop development (i) at the vegetative stages prior to the transition to reproductive —commonly referred to as 'winter hardiness'; and (ii) at anthesis—commonly referred to as 'reproductive frost tolerance'. The focus of this report is on the former; for a review of the latter, see Frederiks et al. (2012).

Barley (*Hordeum vulgare* L.) is an economically important model system for the Triticeae in many contexts, including winter hardiness (reviewed in Muñoz-Amatriain et al. 2014a). Remarkable progress has been made in sequencing the barley genome (e.g. International Barley Genome Sequencing Consortium 2012, Mascher et al. 2013, Muñoz-Amatriain et al. 2015), and several tools such as BARLEYMAP (Cantalapiedra et al. 2015) and BARLEX (Colmsee et al. 2015) are being developed to assist users in accessing the barley genome. However, winter hardiness is a complex genetic trait

Making an isogenic line

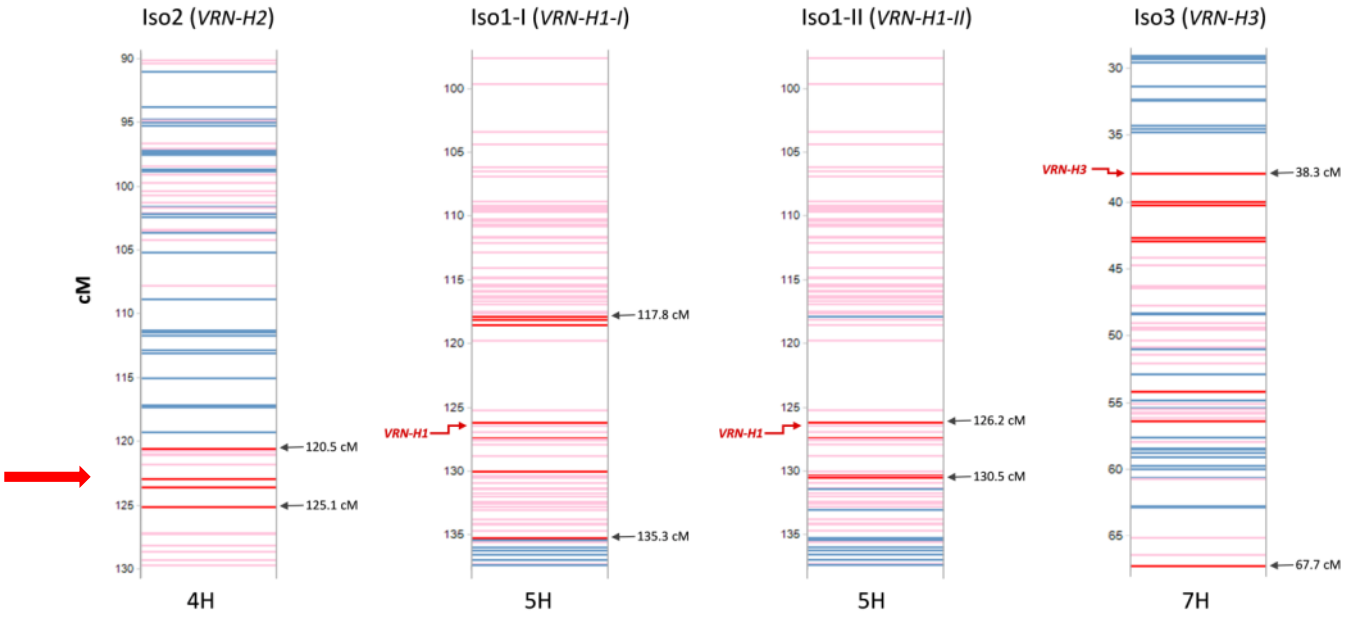
Takahashi and Yasuda created the multiple barley vernalization isogenic lines with 11 backcrosses!



Generation	F1	BC1	BC2	BC5	BC6
Recurrent Parent	50%	75%	87.5%	98.4%	99.2%

http://themadvirologist.blogspot.com/2017/01/what-is-isogenic-line-and-why-should-it.html

Graphical SNP genotypes for the single locus VRN isogenic lines
Blue = recurrent parent; red = donor parent ; pink = monomorphic SNPs



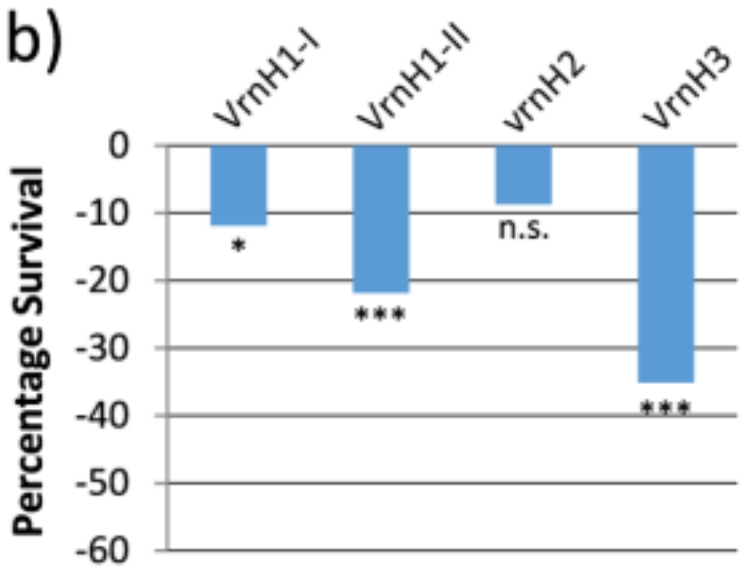
- Map-ordered SNPs reveal defined introgressions on target chromosomes
- Alignment with genome sequence allows estimates of gene number and content within introgressions
- Estimate genetic (5 – 30 cM) and physical (7 – 50 Mb) sizes of introgressions

Gene annotations for the *VRN-H2* genes present in the winter parent and absent in the spring donor (deletion allele)

morex_contig_139172	4	113.56	MLOC_7409.1	HC	Small nuclear ribonucleoprotein-like protein
morex_contig_326580	4	113.56	MLOC_48605.2	LC	-
morex_contig_326580	4	113.56	MLOC_48606.1	LC	-
morex_contig_326580	4	113.56	MLOC_48604.1	LC	-
morex_contig_326580	4	113.56	MLOC_48603.3	LC	-
morex_contig_44376	4	113.56	MLOC_60425.1	HC	UPF0187-containing protein
morex_contig_44376	4	113.56	MLOC_60426.3	HC	RNA polymerase II transcription mediators LENGTH=2253
morex_contig_1705101	4	113.74	MLOC_25653.1	LC	-
morex_contig_171282	4	113.74	MLOC_25773.1	HC	rRNA N-glycosidase
morex_contig_190940	4	113.74	MLOC_28335.1	LC	-
morex_contig_203313	4	113.74	MLOC_29747.2	LC	-
morex_contig_45870	4	113.74	AK372562	HC	60 kDa jasmonate-induced protein, putative
morex_contig_48791	4	113.74	MLOC_64410.1	LC	-
morex_contig_52290	4	113.74	MLOC_66920.1	LC	-
morex_contig_62907	4	113.74	AK368847	HC	Protein of unknown function (DUF3527) LENGTH=694
morex_contig_1559316	4	114.94	MLOC_10539.3	HC	ABC(ATP-binding) family transporter
morex_contig_1566159	4	114.94	AK365195	HC	basic helix-loop-helix (bHLH) DNA-binding superfamily protein LENGTH=264
morex_contig_1566323	4	114.94	MLOC_13712.1	LC	-
morex_contig_1566323	4	114.94	MLOC_13711.1	LC	-
morex_contig_1566323	4	114.94	MLOC_13710.1	LC	-
morex_contig_1569145	4	114.94	AK371802	HC	Cytochrome P450 cinnamate 4-hydroxylase
morex_contig_1574321	4	114.94	MLOC_16737.1	HC	AT hook motif DNA-binding protein
morex_contig_1583381	4	114.94	MLOC_19070.1	LC	-
morex_contig_1583518	4	114.94	MLOC_19112.1	LC	-
morex_contig_160316	4	114.94	MLOC_22160.1	HC	HXXXD-type acyl-transferase family protein LENGTH=428
morex_contig_2199658	4	114.94	MLOC_31549.1	LC	-
morex_contig_268140	4	114.94	MLOC_43160.1	LC	-
morex_contig_438357	4	114.94	AK251005.1	HC	Tubulin beta chain, putative
morex_contig_47845	4	114.94	MLOC_63681.2	LC	-
morex_contig_6397	4	114.94	MLOC_73586.1	LC	-
morex_contig_64203	4	114.94	AK248746.1	HC	basic helix-loop-helix (bHLH) DNA-binding superfamily protein LENGTH=181
morex_contig_7732	4	114.94	AK361249	HC	Pentatricopeptide repeat-containing protein, putative
morex_contig_123975	4	115.01	MLOC_2684.1	LC	-
morex_contig_1558926	4	115.01	MLOC_10370.1	LC	-
morex_contig_1604879	4	115.01	MLOC_22283.1	LC	-
morex_contig_2548710	4	115.01	AK375557	HC	Phosphoglycerate mutase family protein
morex_contig_46264	4	115.01	MLOC_62174.2	LC	-
morex_contig_42417	4	115.08	MLOC_58174.1	LC	-
morex_contig_42417	4	115.08	AK357540	LC	-
morex_contig_42417	4	115.08	AK368048	LC	-
morex_contig_42417	4	115.08	MLOC_58176.2	HC	Protein kinase superfamily protein LENGTH=824
morex_contig_42417	4	115.08	MLOC_58173.1	LC	-
morex_contig_42417	4	115.08	MLOC_58177.1	LC	-
morex_contig_1584974	4	115.23	MLOC_19449.2	LC	-
morex_contig_37692	4	115.23	MLOC_52663.4	HC	AP-2 complex subunit alpha, putative

17 predicted genes

- No flowering time or low temperature tolerance–related genes in the *VRN-H2* introgression
- Can we therefore have the *VRN-H2* deletion and maintain cold tolerance?



No significant loss in low temperature tolerance with the *VRN-H2* deletion

VRN allele architecture, vernalization sensitivity and low temperature tolerance

*VRN-H_* loci and allelic configurations			Vernalization sensitivity	Low temperature tolerance
V1	***V2***	***V3***		
V	V	V	No	No
V	V	*v*	No	No
V	*v*	V	No	No
V	*v*	*v*	No	No
v	V	V	No	No
v	V	*v*	Yes	Yes
v	*v*	V	No	No
v	*v*	*v*	No	Yes

7:1 ratio (DH)

Takahashi and Yasuda (1971)
Cuesta-Marcos et al. (2015)

Facultative
Growth habit

Climate change: Facultative growth habit

Fall planting
Cold tolerance
on demand

Spring planting
Cold tolerance
not needed

Facultative growth habit – are you ready for THE CHANGE?

How?

- "Just say no" to vernalization sensitivity with the "right" *VRN-H2* allele
  - A complete deletion
- "Just say yes" to short day photoperiod sensitivity with the "right" photoperiod sensitivity allele (*PPD-H2)*
- "Ensure" a winter haplotype at all low temperature tolerance loci
  - *Fr-H1, FR-H2*, and *FR-H3* plus…. a continual process of discovery

Necessary parameters for genetic analysis

Mendelian genetic analysis: the "classical" approach to understanding the genetic basis of a difference in phenotype is to use progeny to understand the parents.

- *If you use progeny to understand parents, then you make crosses between parents to generate progeny populations of different filial (F) generations: e.g. F1, F2, F3; backcross; doubled haploid; recombinant inbred, etc.*

Necessary parameters for genetic analysis

Mendelian genetic analysis: the "classical" approach to understanding the genetic basis of a difference in phenotype is to use progeny to understand the parents.

- *The genetic status (degree of homozygosity) of the parents will determine which generation is appropriate for genetic analysis and the interpretation of the data (e.g. comparison of observed vs. expected phenotypes or genotypes).*
    - *The degree of homozygosity of the parents will likely be a function of their mating biology, e.g. cross vs. self-pollinated.*

Necessary parameters for genetic analysis

Mendelian genetic analysis: the "classical" approach to understanding the genetic basis of a difference in phenotype is to use progeny to understand the parents.

- *Mendelian analysis is straightforward when one or two genes determine the trait.*
- *Expected and observed ratios in cross progeny will be a function of*
    - *the degree of homozygosity of the parents*
    - *the generation studied*
    - *the degree of dominance*
    - *the degree of interaction between genes*
    - *the number of genes determining the trait*