WGIN Resource Development

Simon Griffiths

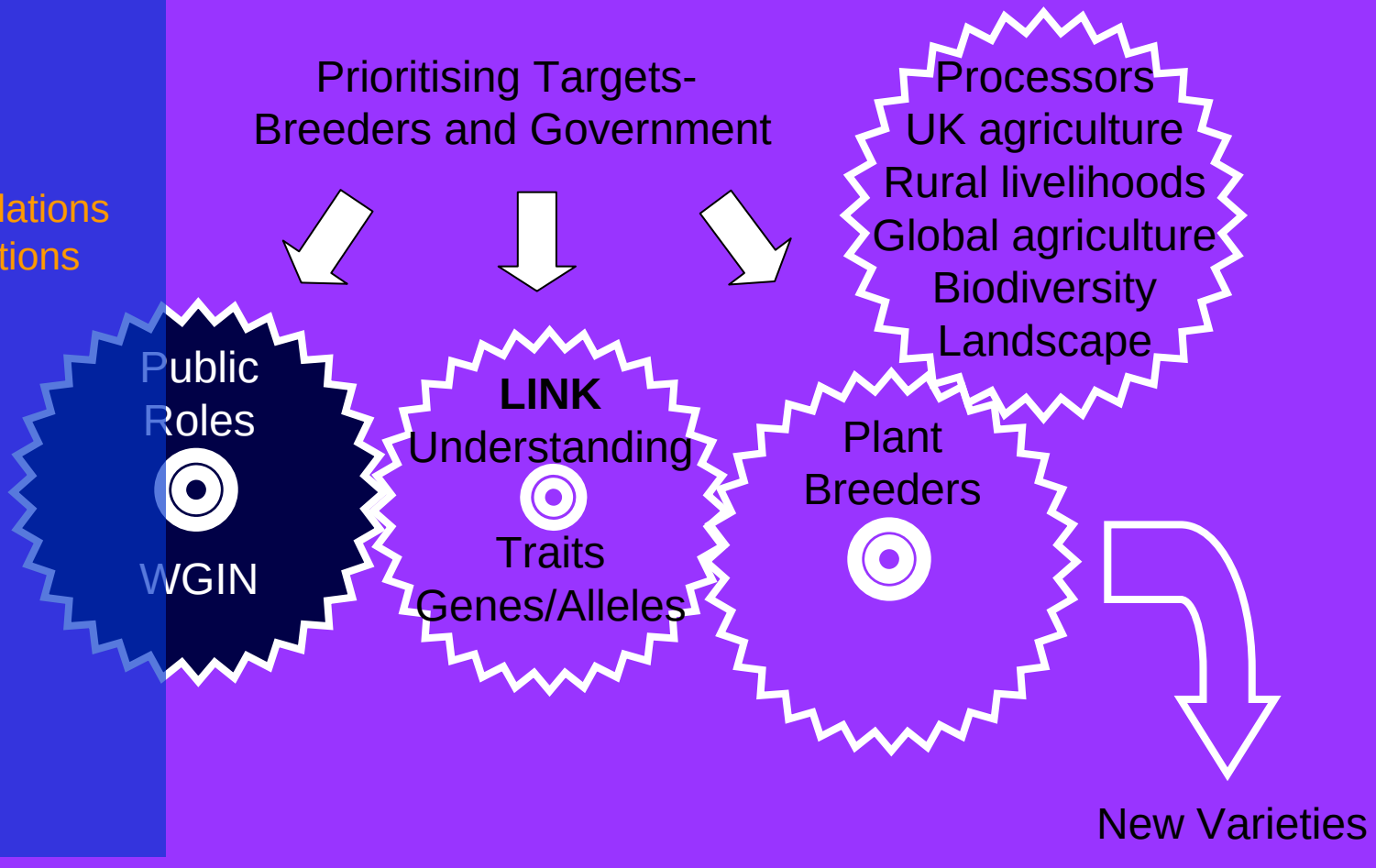
Delivery of genetic improvement in wheat- The role of UK science

Genetics
Genetic analysis
Positional Cloning
Mutants

Germplasm
Collections
Mapping populations
Mutant populations

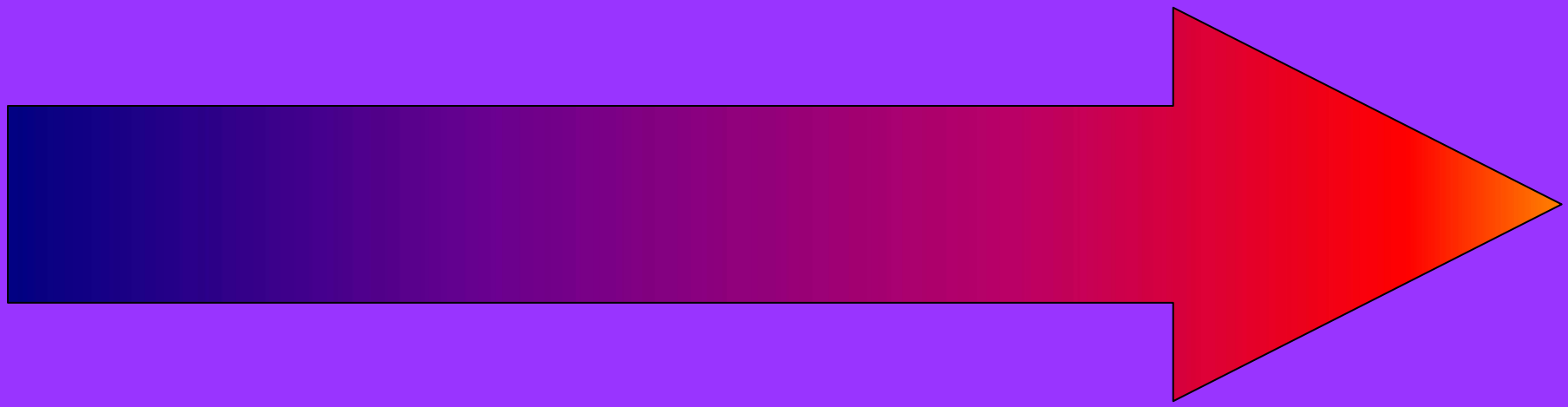
Genomics
Bioinformatics
Markers
Transcripts
Targeted mutagenesis

Mechanisms
How do genes change phenotype?



The challenge of exploiting and understanding genetic diversity

Elite hexaploids	Landraces & old varieties	Induced allelic variation	Wheat relatives
Avalon x Cadenza	AE Watkins and Grdiflux	EMS and gamma mutation	*T monococcum* work at RRes



Success of WGIN resource development

1. The hidden work- Seed stock/DNA maintenance , curation, and distribution for Avalon x Cadenza, Paragon EMS, Paragon gamma, Gediflux, and Watkins.

2. Added value of research which then facilitates the exploitation of these resources.

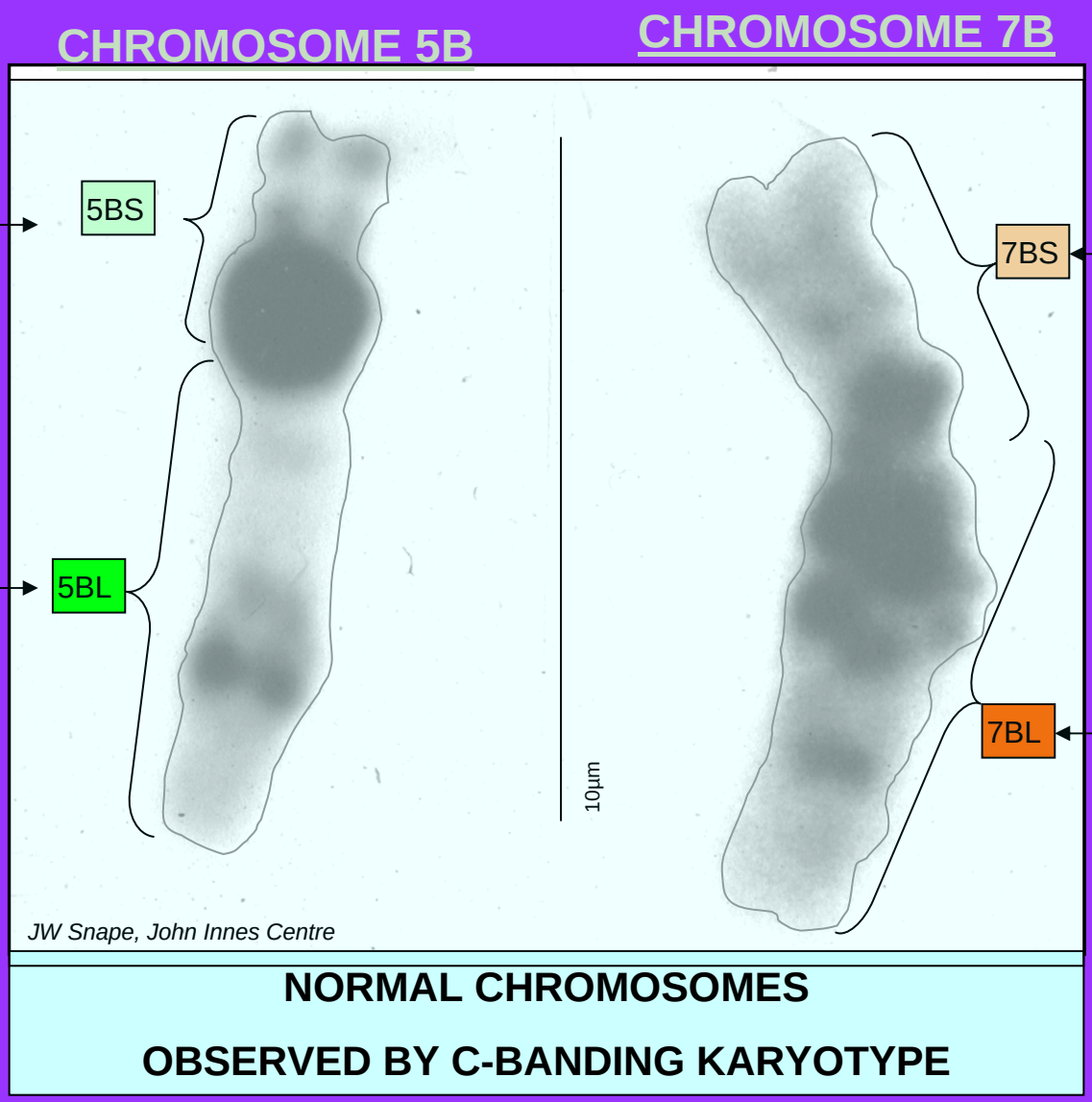
Avalon x Cadenza

Mapping

Avalon x Cadenza

- Doubled haploid population of 204 individuals
- Major effects: *Vrn-A*1 and *Rht-D*1
- Other factors: 5B-7B translocation

HEXAPLOID WHEAT
CULTIVAR CADENZA



NORMAL CHROMOSOMES

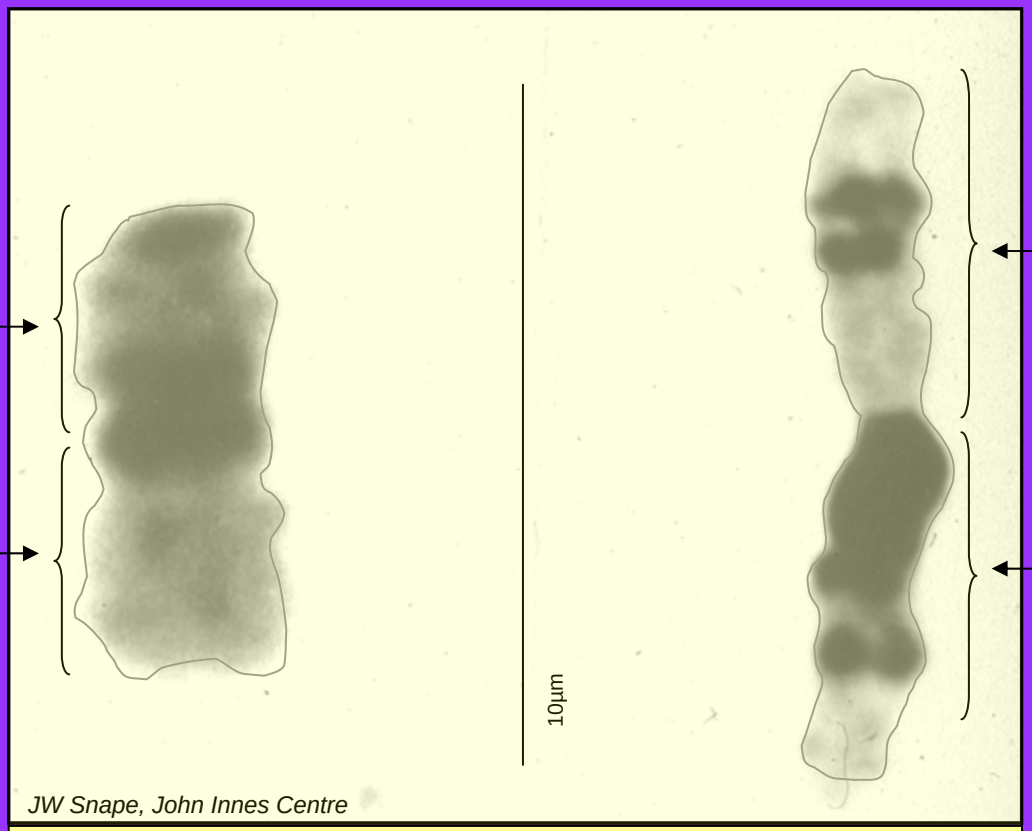
OBSERVED BY C-BANDING KARYOTYPE

HEXAPLOID WHEAT
CULTIVAR MARIS PLOUGHMAN

The 2 chromosomes show a chromosome rearrangement called a "**translocation**". The short arm of chromosome 5B is pairing with the short arm of chromosome 7B. The same happens with the long arms.

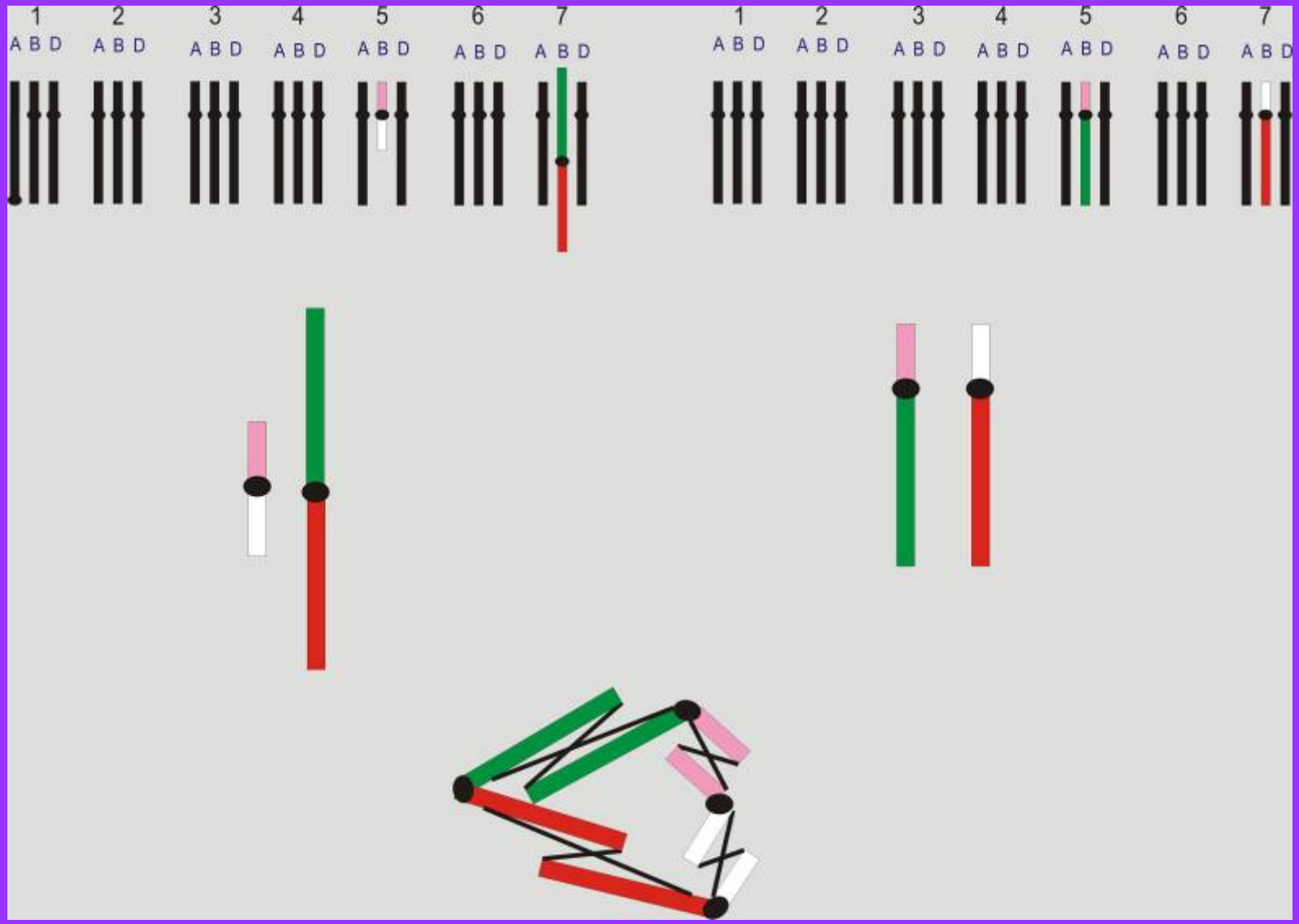
CHROMOSOME 5BS/7BS

CHROMOSOME 5BL/7BL



TRANSLOCATED CHROMOSOMES, DETECTED BY C-BANDING KARYOTYPE

Accounting for the presence of 5B-7B translocation in Avalon



Cytogenetic analysis of Avalon x Cadenza population

- C-banding carried out by Adam Lukaszewski (Riverside)
- 87 of 204 lines contain 5B-7B
- Some surprises- DH4 appears to be 1BL-1RS!
- In the process of remaking map with translocation + and – lines separated.

Gene based markers

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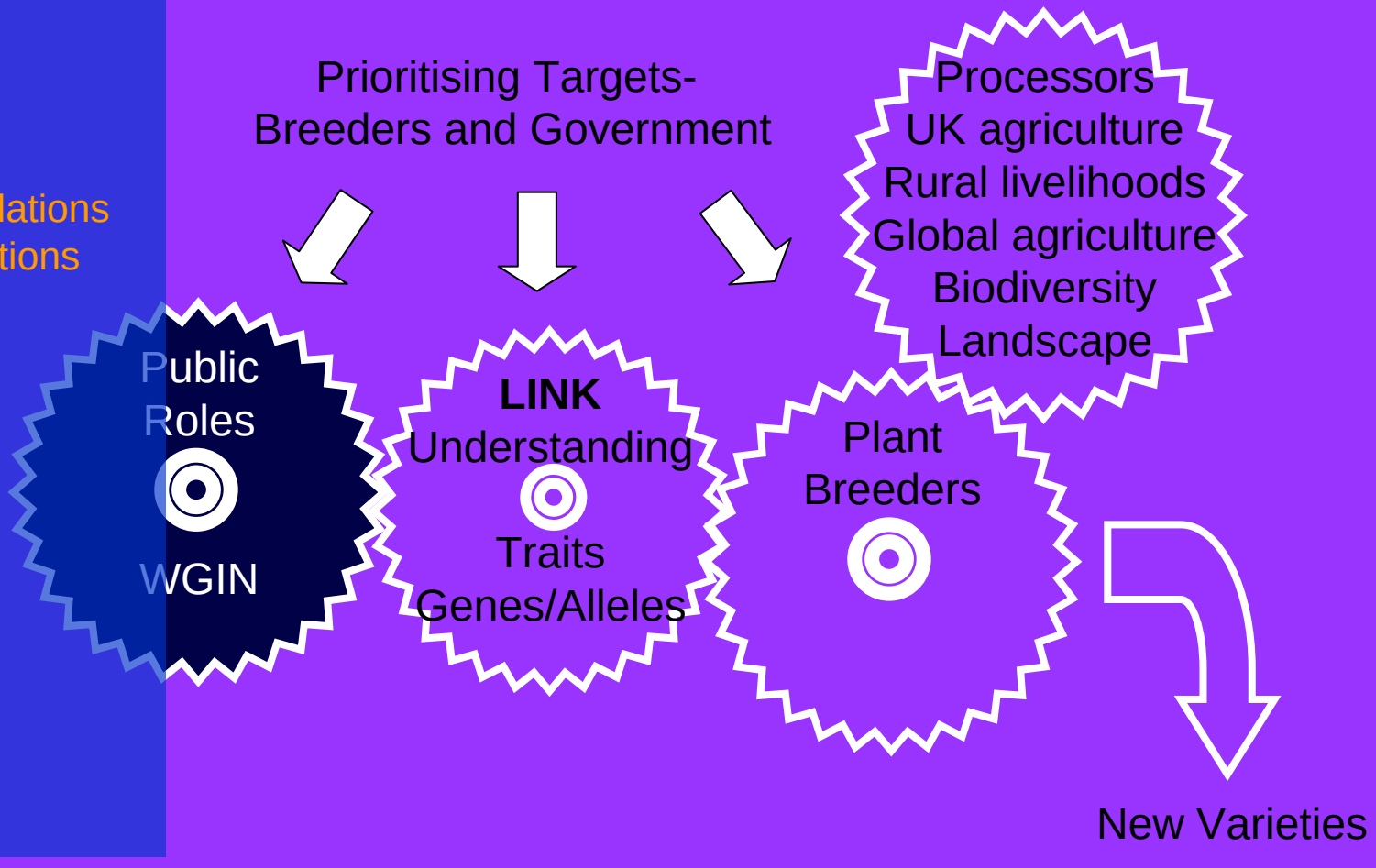
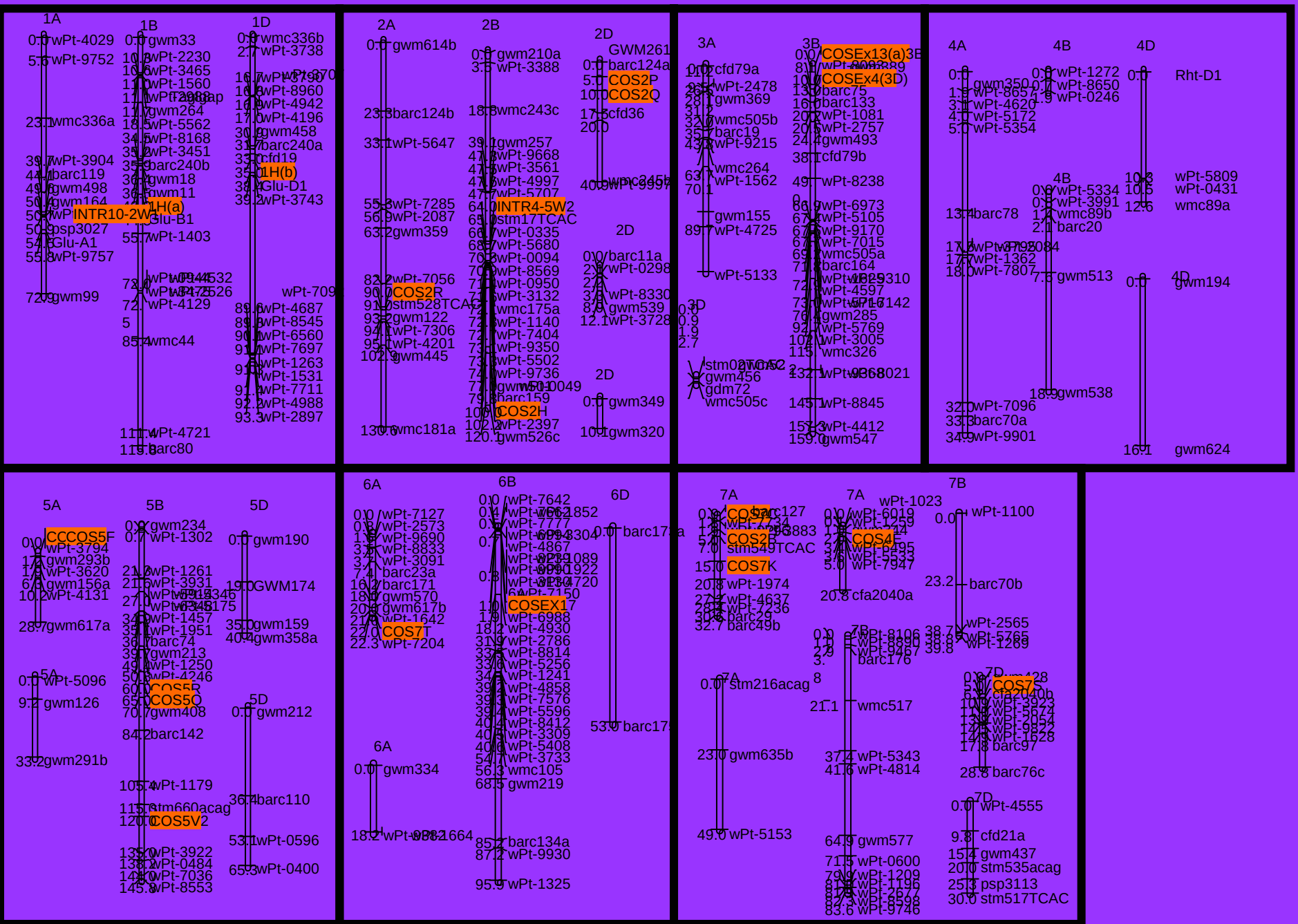
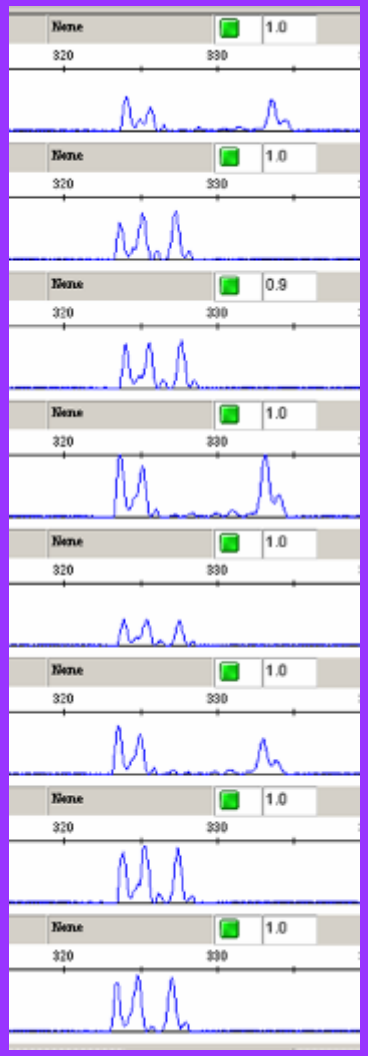
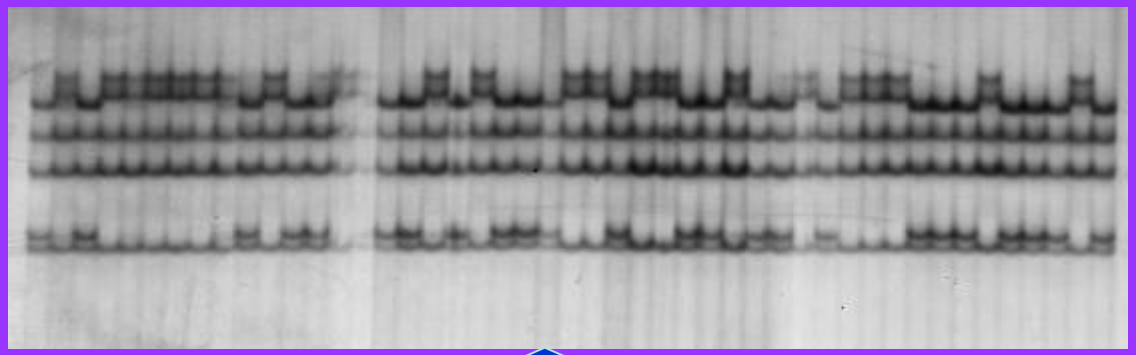


Gene based markers on Avalon X Cadenza map



Single Strand Conformation Polymorphism is now a high throughput technique





Conventional SSCP ran on non-denaturing acrylamide

ABI3730 capillary electrophoresis (modified polymer)

More Gene Based Markers from Tools and Resources Project

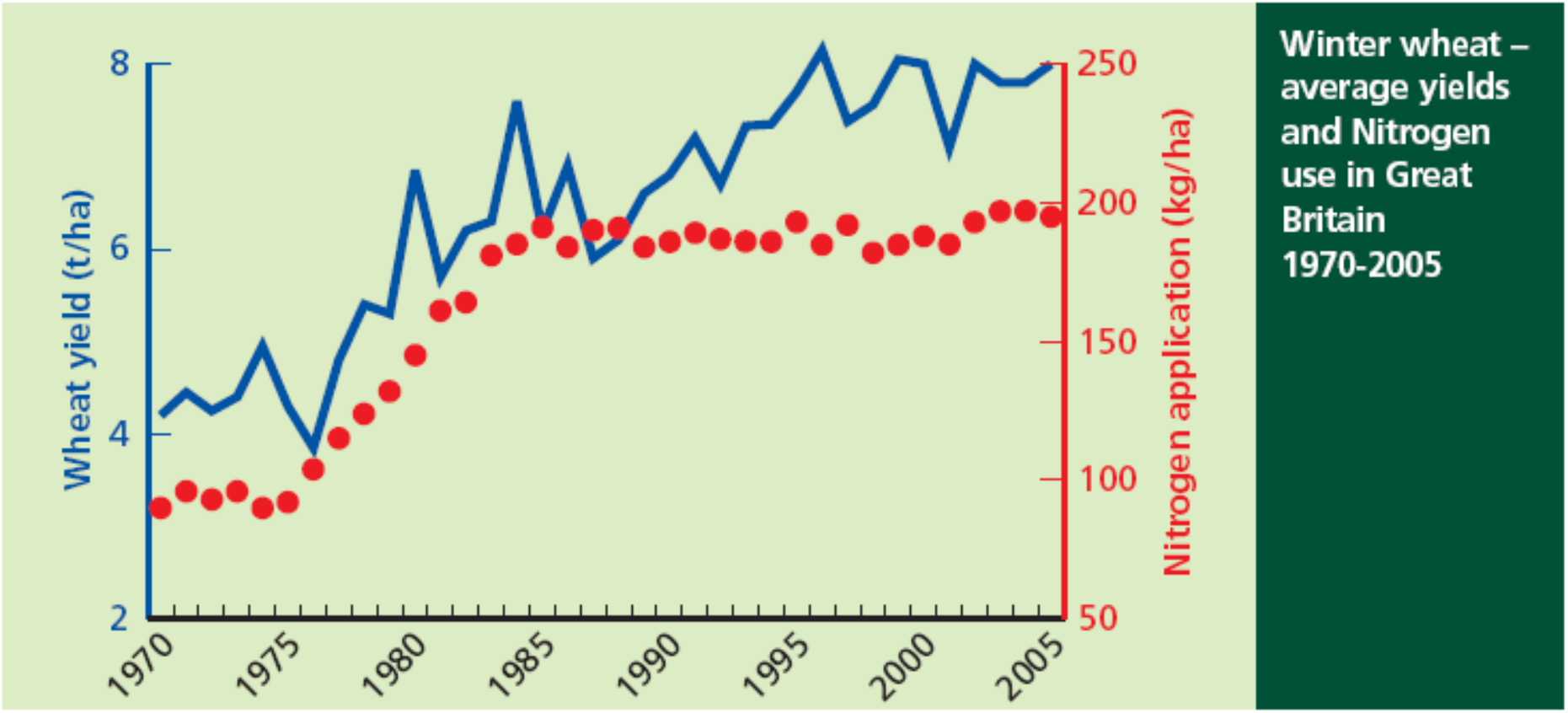
- So far, 830 primer pairs screened on Avalon x Cadenza and Opata x Synthetic. A further 110 will be tested after next mapping phase.
- High quality, low copy, amplification in over 95% of cases.
- Polymorphism lower than expected based on WGIN acrylamide set.
- 10% in A x C and 15% in O x S.
- Project will deliver ~100 new mapped genes in both populations.
- Sufficient funds to search for more in extra populations.

Two new SSD populations now up F_3
Paragon x Chinese Spring and Paragon x JIC synthetic

Avalon x Cadenza

Phenotyping and QTL analysis

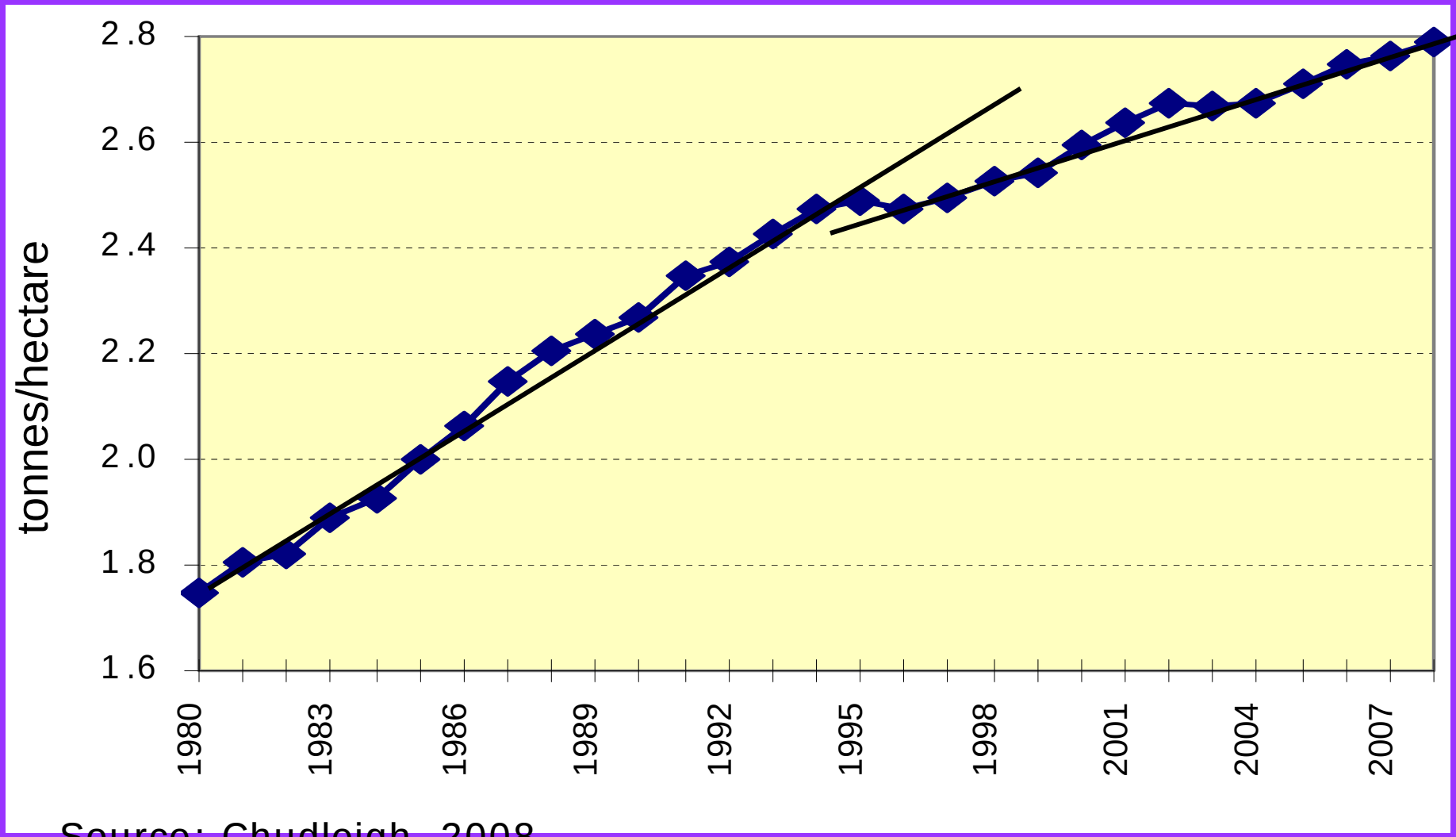
Recent improvements in UK yield and quality have occurred against a background of stable N application



Winter wheat – average yields and Nitrogen use in Great Britain 1970-2005

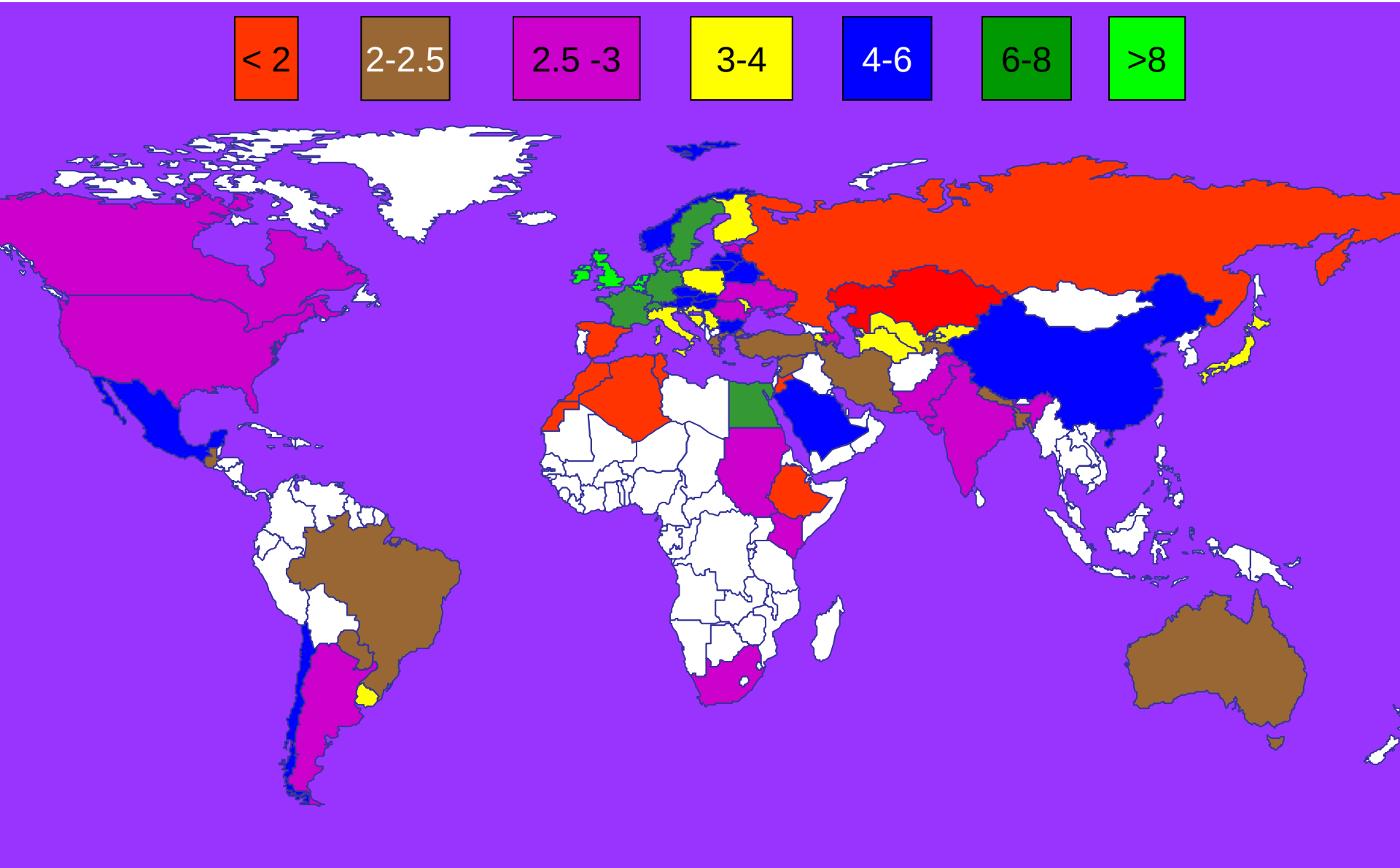
WORLD WHEAT YIELD TRENDS

(5 Year Moving Average - tonnes/ha)

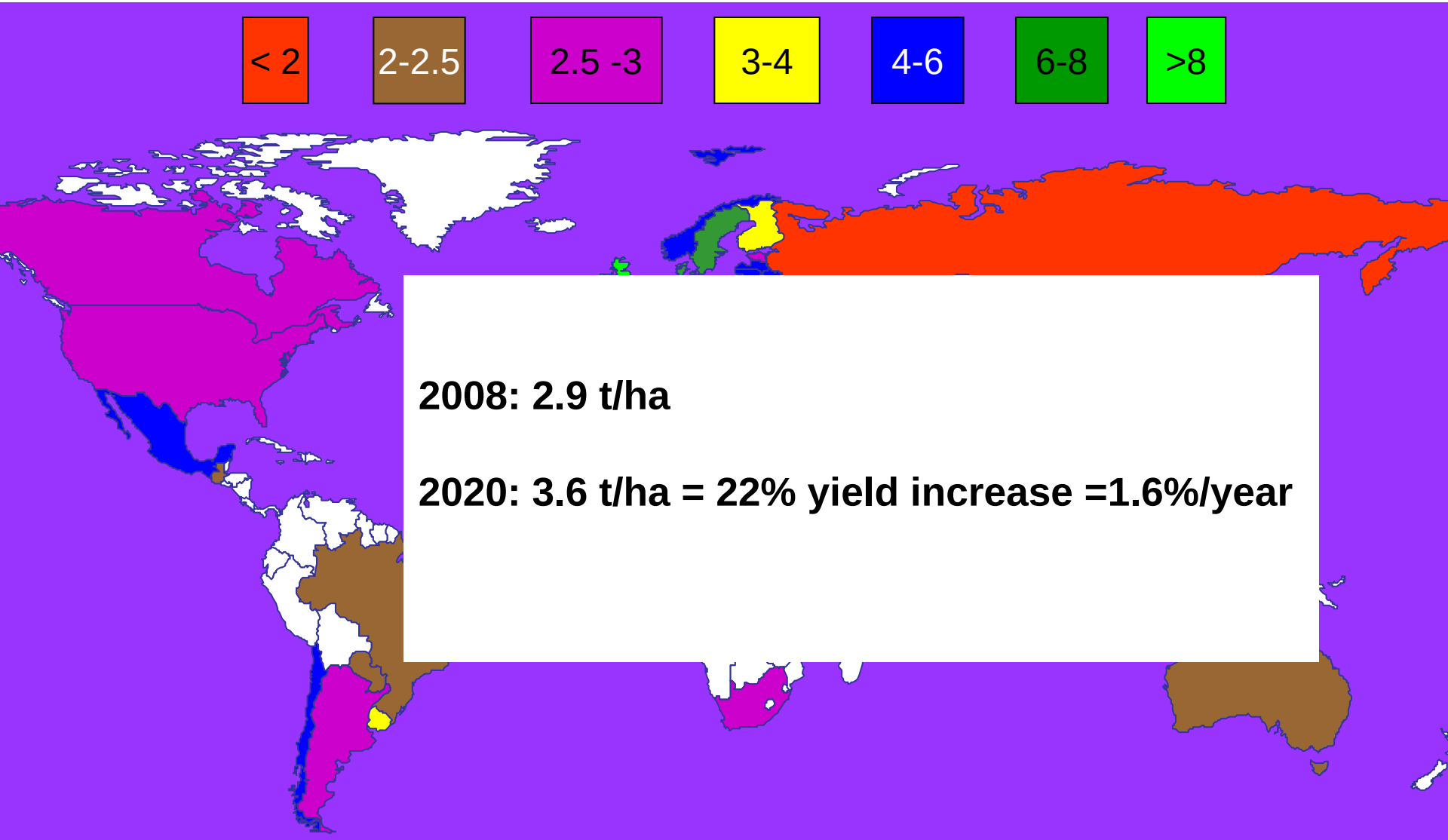


Source: Chudleigh, 2008

Average Wheat Yield of countries producing more than 300 000 tons



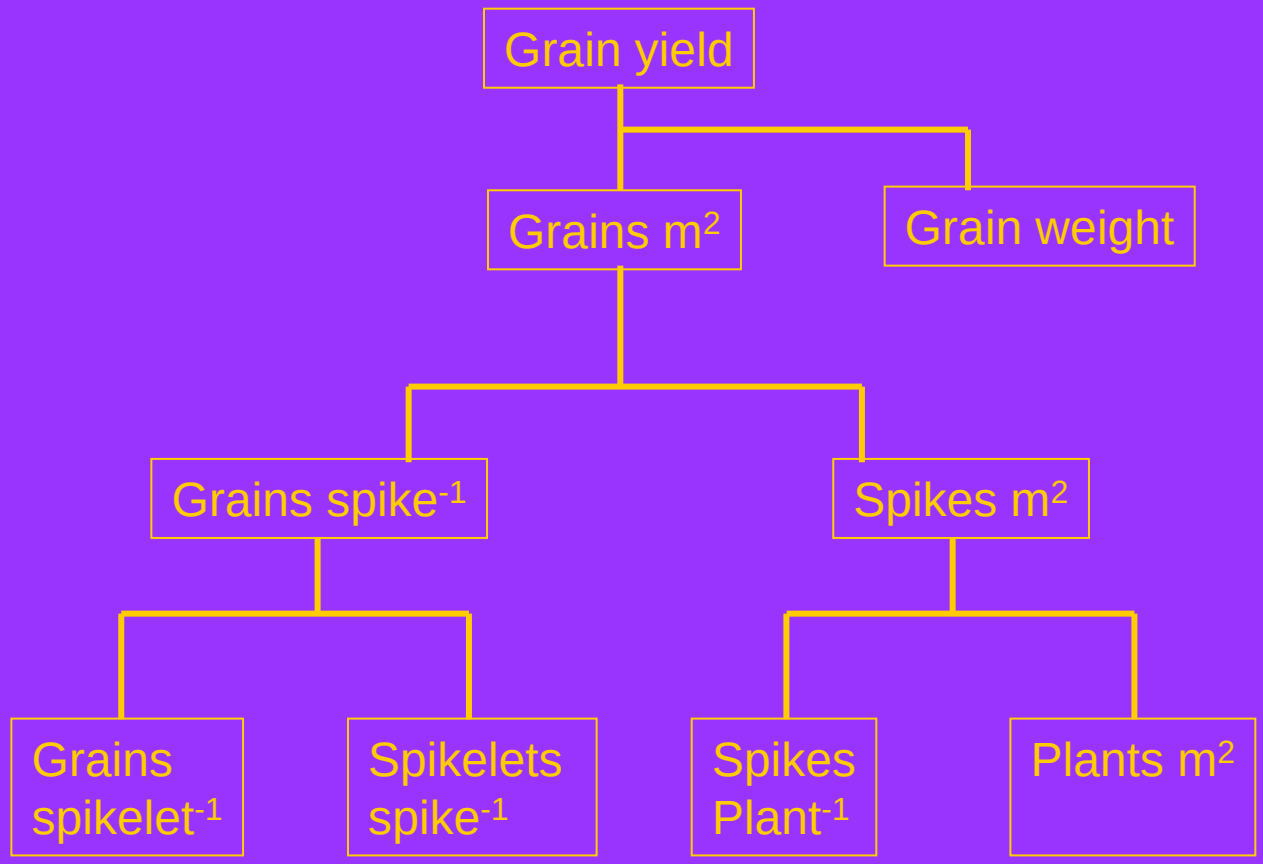
Average Wheat Yield of countries producing more than 300 000 tons



2008: 2.9 t/ha

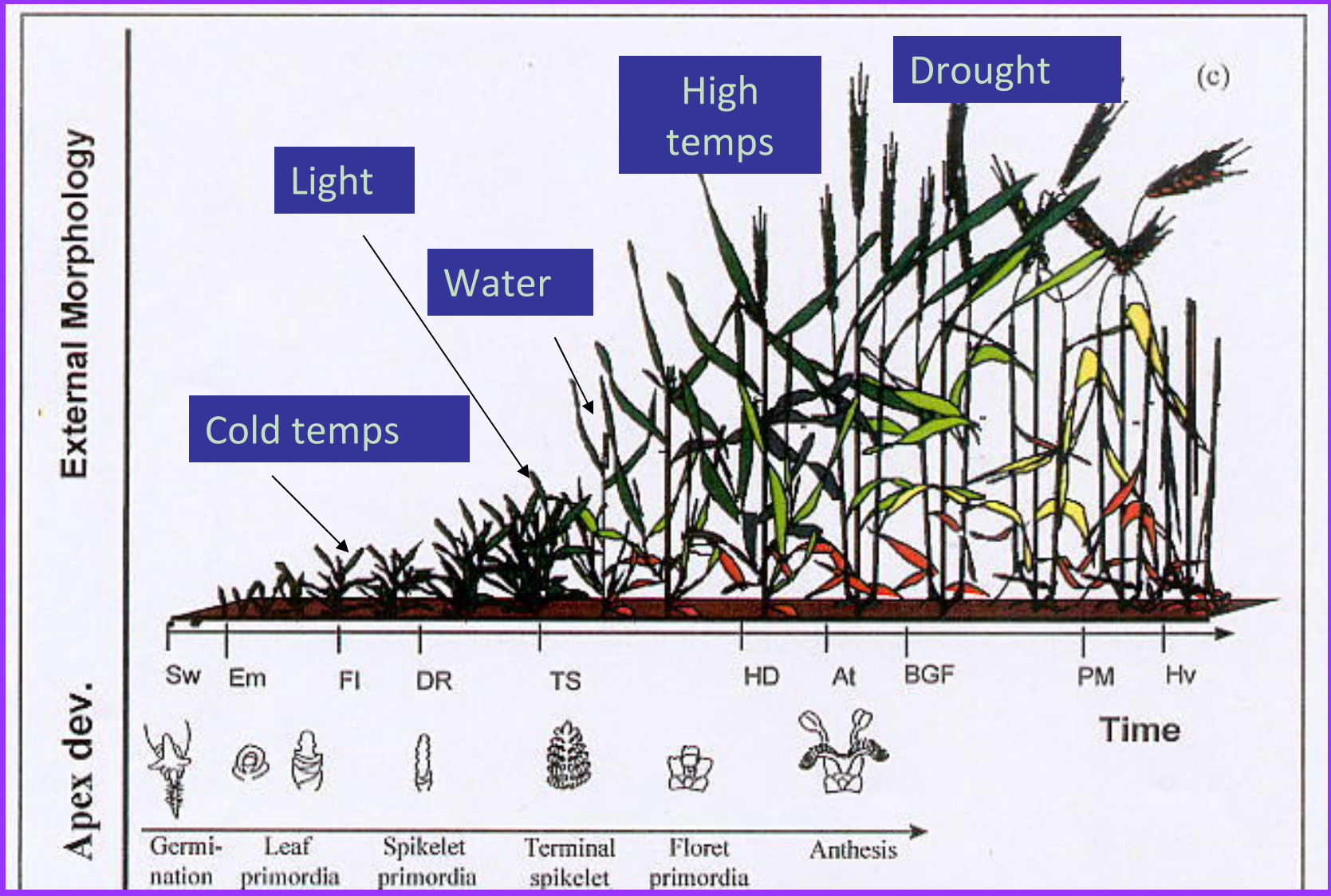
2020: 3.6 t/ha = 22% yield increase =1.6%/year

Identification of genetic variation for grain yield potential deployed by UK wheat breeders



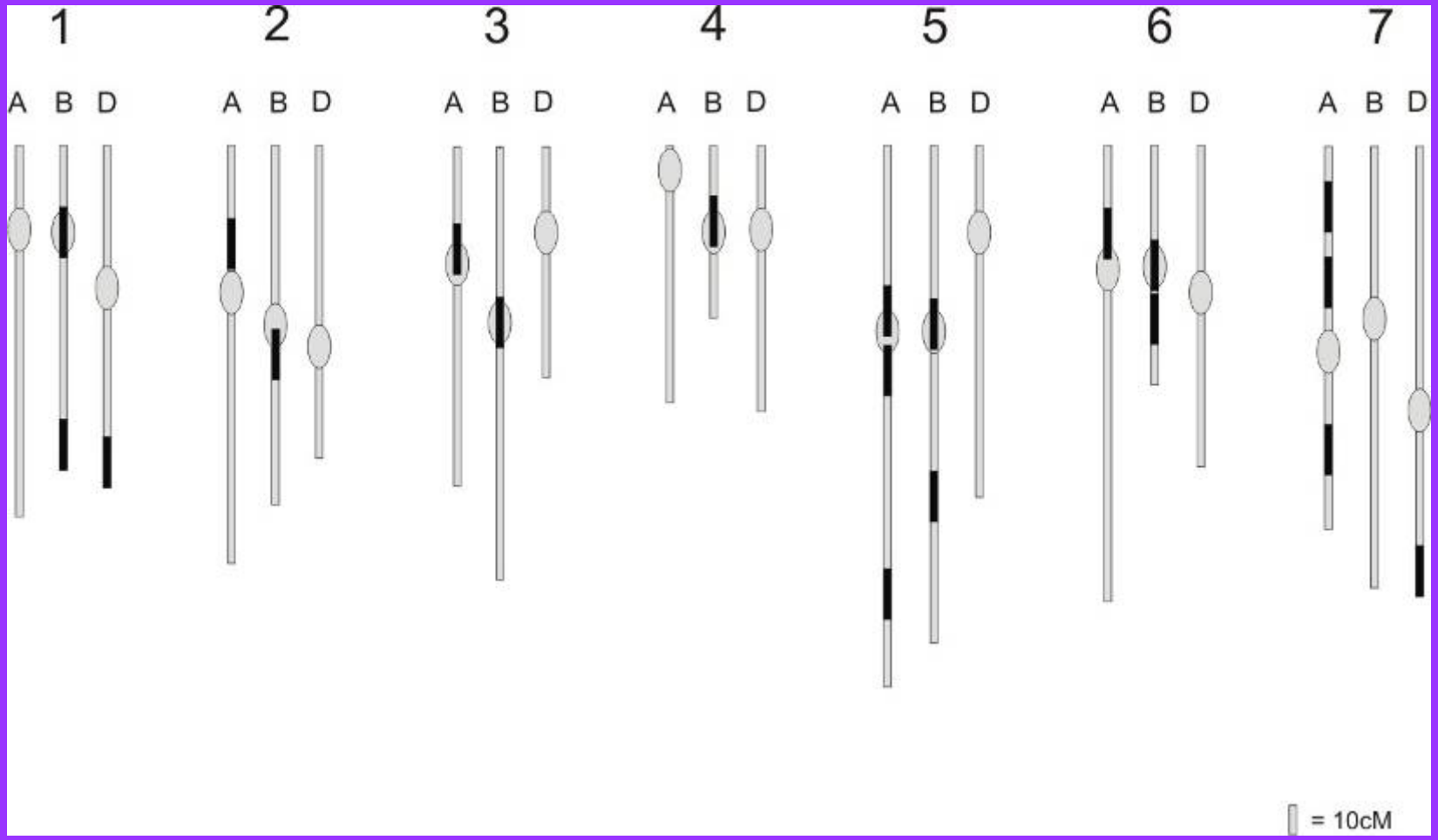
All these components available for Avalon x Cadenza population for three years.

Importance of floral development and plant architecture for resource capture and utilisation



(after Slafer & Rawson, 1994)

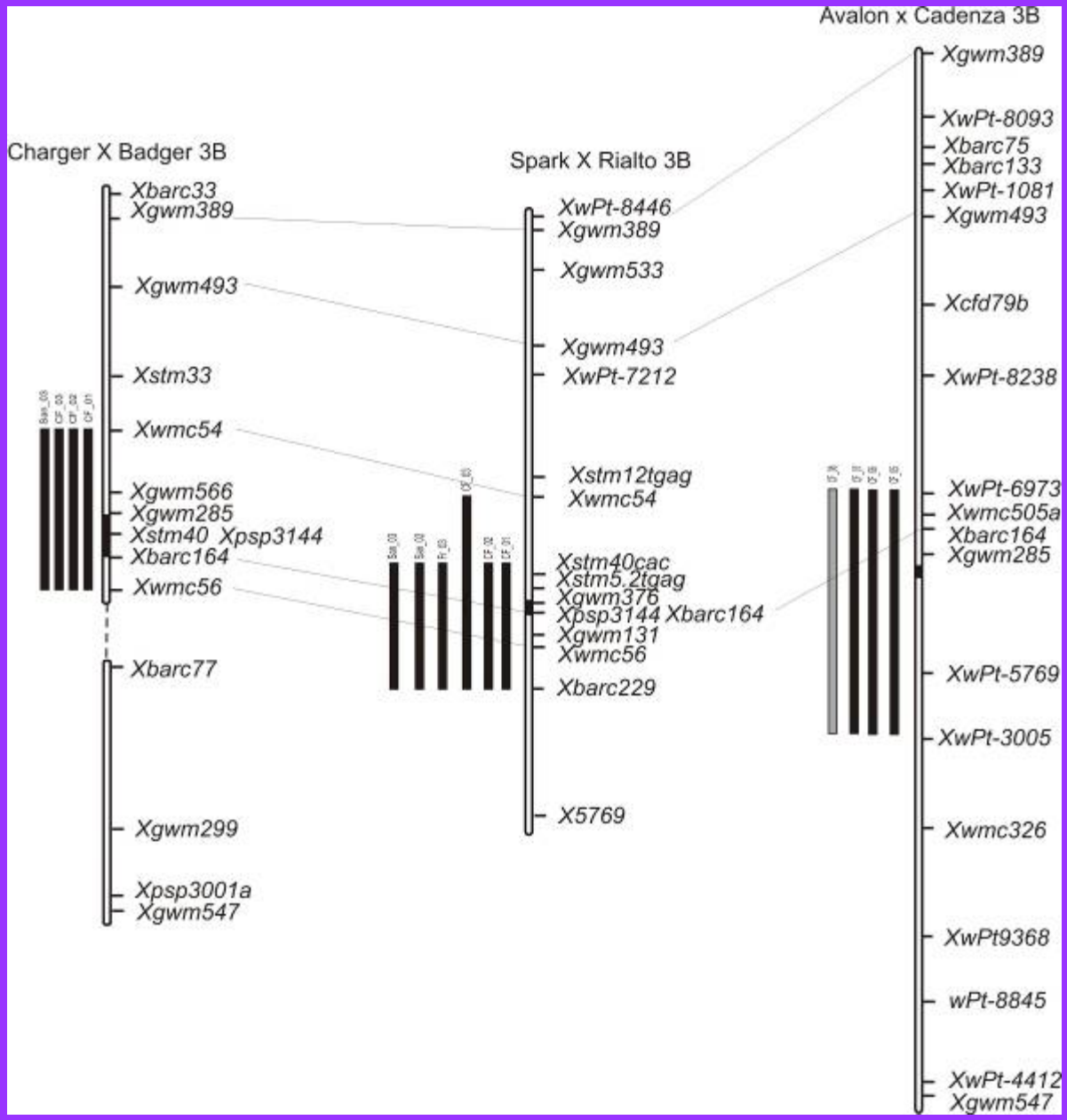
Summary of data using consensus map and meta analysis, ear emergence example:



Where do we go with our QTL?

- Within very recent pedigrees MAS will work well.
- Cloning genes is essential for MAS to really work ie selecting directly for the functional polymorphism and understanding how the gene works.
- WGIN has put tools in place to facilitate dissection of QTL.

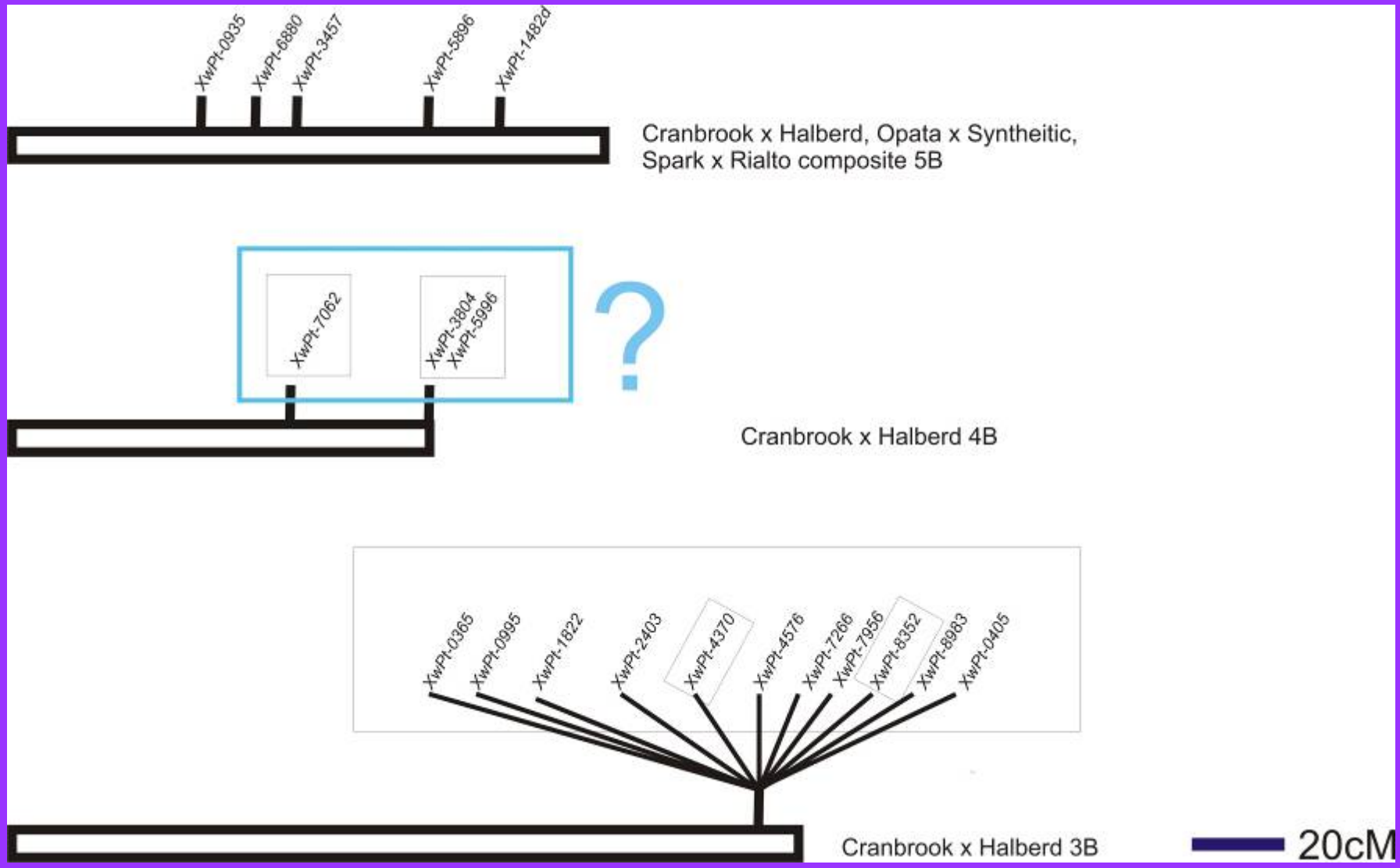
Location of 3B height effects identified in three DH populations



Characterising deletions using DArT

- 94 Paragon gamma deletion lines.
- 13000 features on the high density array.
- 554 clear deletions detected in replicated (x2) hybridisation.
- Represents 4.3 % of all DArT features.
- Would 2500 approach good genome coverage?

We can dissect QTL and produce physical maps using the WGIN gamma deletion set.



Novel genetic variation from Watkins and Gediflux material

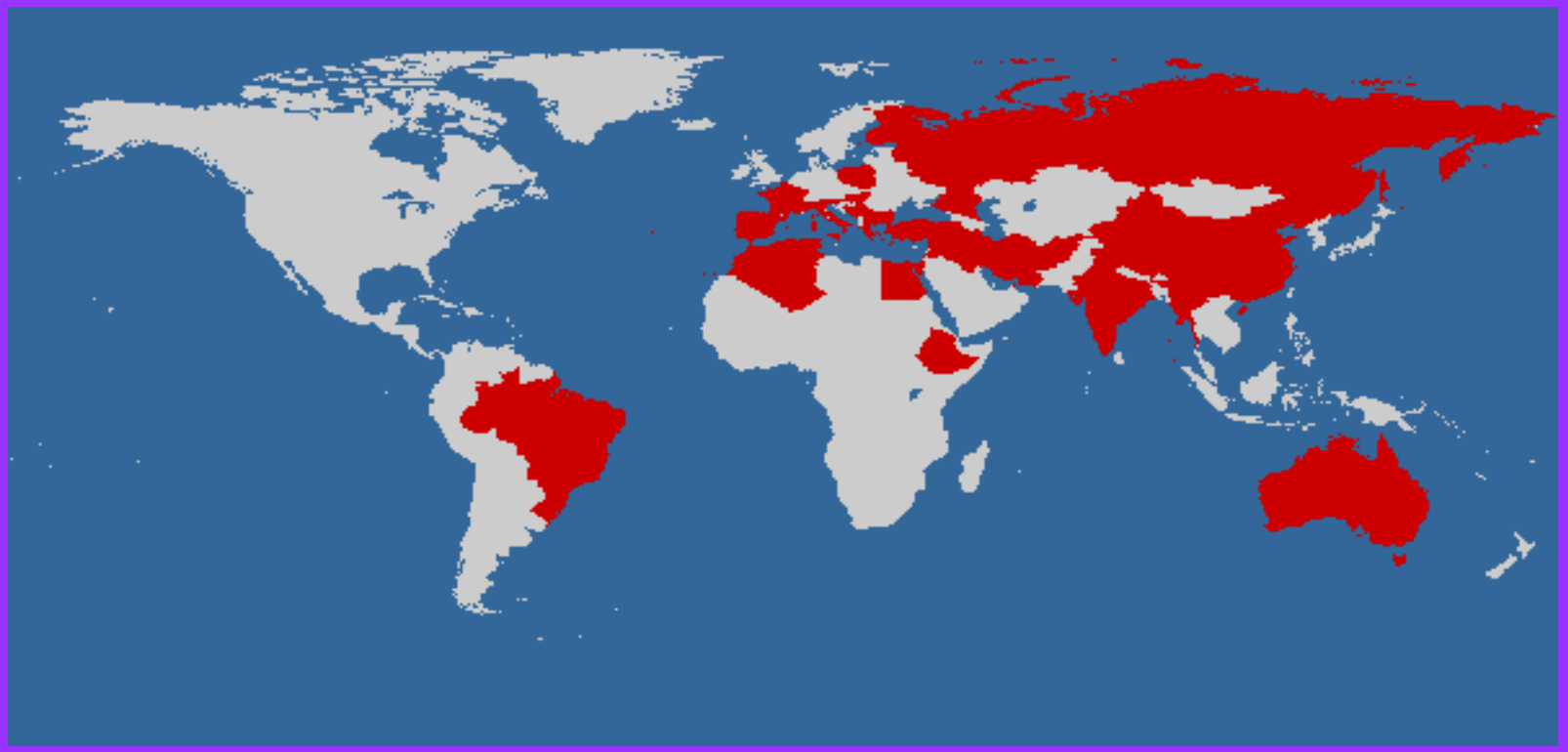
GEDIFLUX Collection

- 510 Northern European varieties deemed important from 1940 – 2000
- Genotyped for an EU diversity project using SSR microsats, retrotransposons elements and NBS (disease resistance gene analogues) profiles
- Seed, DNA, genotypic and phenotypic data available
  www.jic.ac.uk/science/CropGen/GEDIFLUX/index.htm
  simon.orford@bbsrc.ac.uk

Watkins Collection

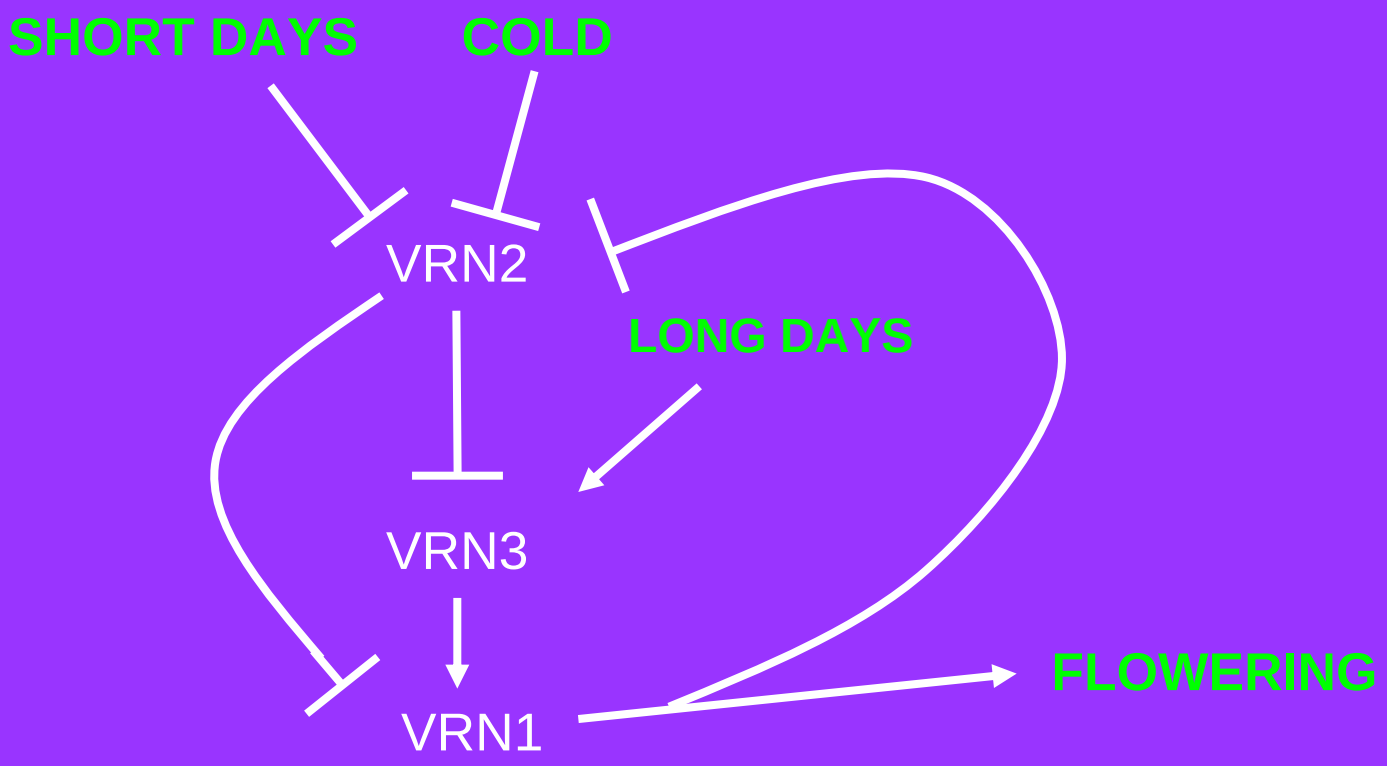
- Collection from markets
- Uniformity within accessions assessed from four rows originating from four individual seed
- Heights, ear emergence, seasonal habit, susceptibilities, waxiness, ear morphology used to assess uniformity

Origins of Watkins Collection

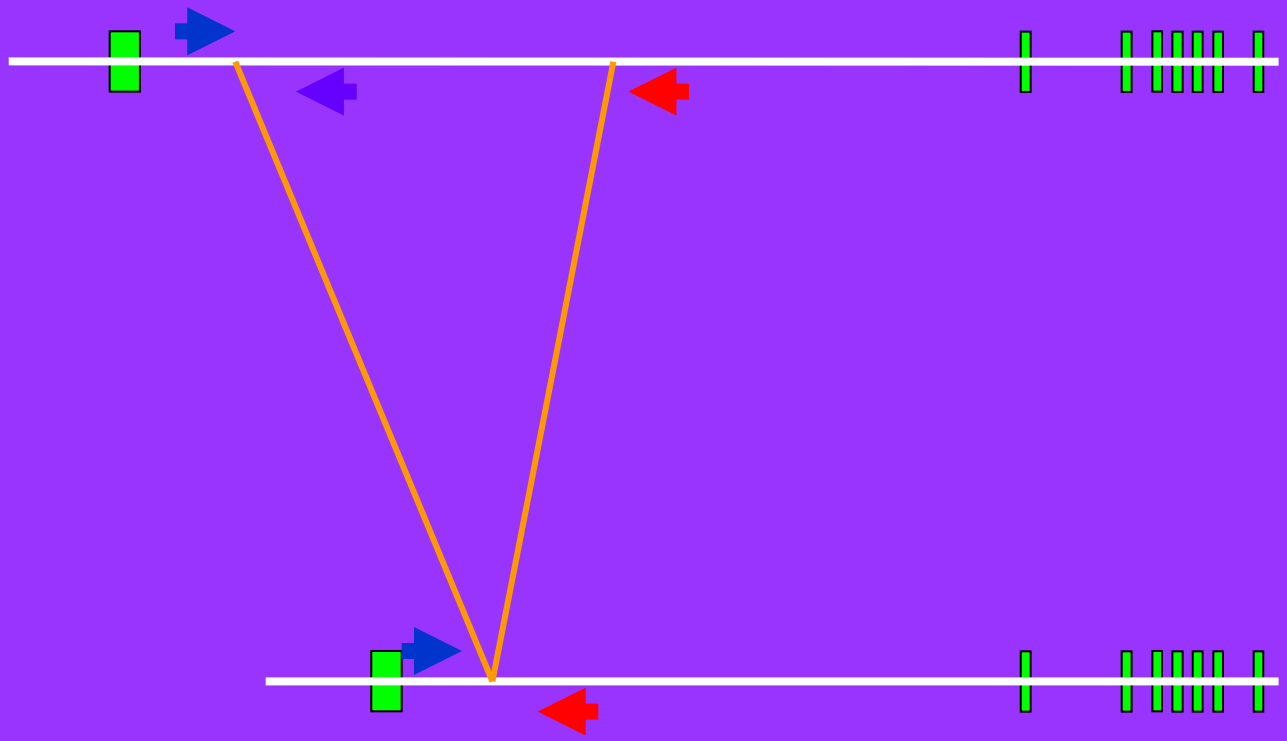


- Africa: Algeria, Canary Islands, Egypt, Ethiopia, Morocco, Tunisia,
- Asia and Middle East: Afghanistan, Burma, China, India, Iran, Iraq, Palestine, Syria, Turkey,
- Europe: Bulgaria, Crete, Cyprus, Finland, France, Greece, Hungary, Italy, Poland, Portugal, Romania, Spain, UK, USSR, Yugoslavia,
- Australia and Brazil

Investigating genetic pathways in wheat- vernalization as an example



Vrn1- Spring or Winter alleles?



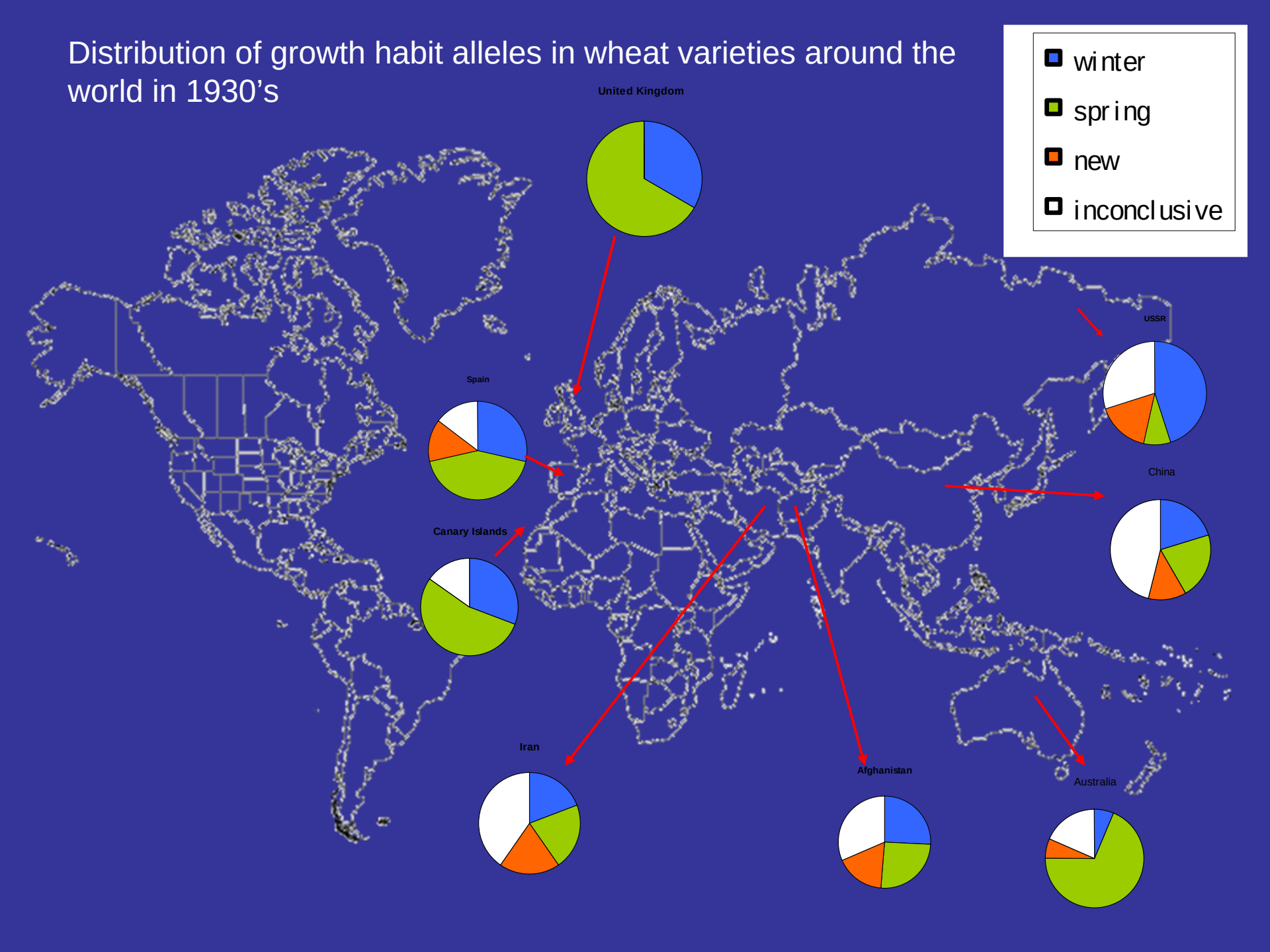
Winter allele-
recessive
wild type

Spring allele-
Dominant mutation

Searching for new genetic variation in response to vernalization

- In a screen of of modern UK spring and alternative wheats- spring habit expalined by known Vrn-A1 promoter duplication and Vrn-B1 intron deletion.
- Screen 800 Watkins lines with all available assays- Do we find spring types that do not have the known alleles?

Distribution of growth habit alleles in wheat varieties around the world in 1930's



New dominant (and recessive?) spring habit genes/alleles from AE Watkins

Mystery spring Watkins x Winter types (eg Alchemy)

Vernalization requirement of F_1?

Map new genes

Sequence new alleles

What if evolution has not provided the allele you are looking for?

New alleles from mutagenesis

- To further characterise a gene of interest
- To generate useful alleles

Paragon Mutant Development

Single Seed Descent (SSD) under glass

Paragon EMS Population

- EMS chemically induced mutations 1% for 16hrs – point mutations.
- Single Seed Descent (SSD) to M_5 generation. Each generation bagged.
- 7000 M_3 plants – 6500 M_6.

EMS Paragon Field Trials

- M_5 seed drilled as 1m rows in field 2006
- Phenotypic notes and photographs taken
- Seed harvested to give stocks for future work
- Specimen ear maintained
- M_6 seed available and M_3 DNA. Check via database www.wgin.org.uk

Paragon EMS examples

Stature

Seed shape

Maturity

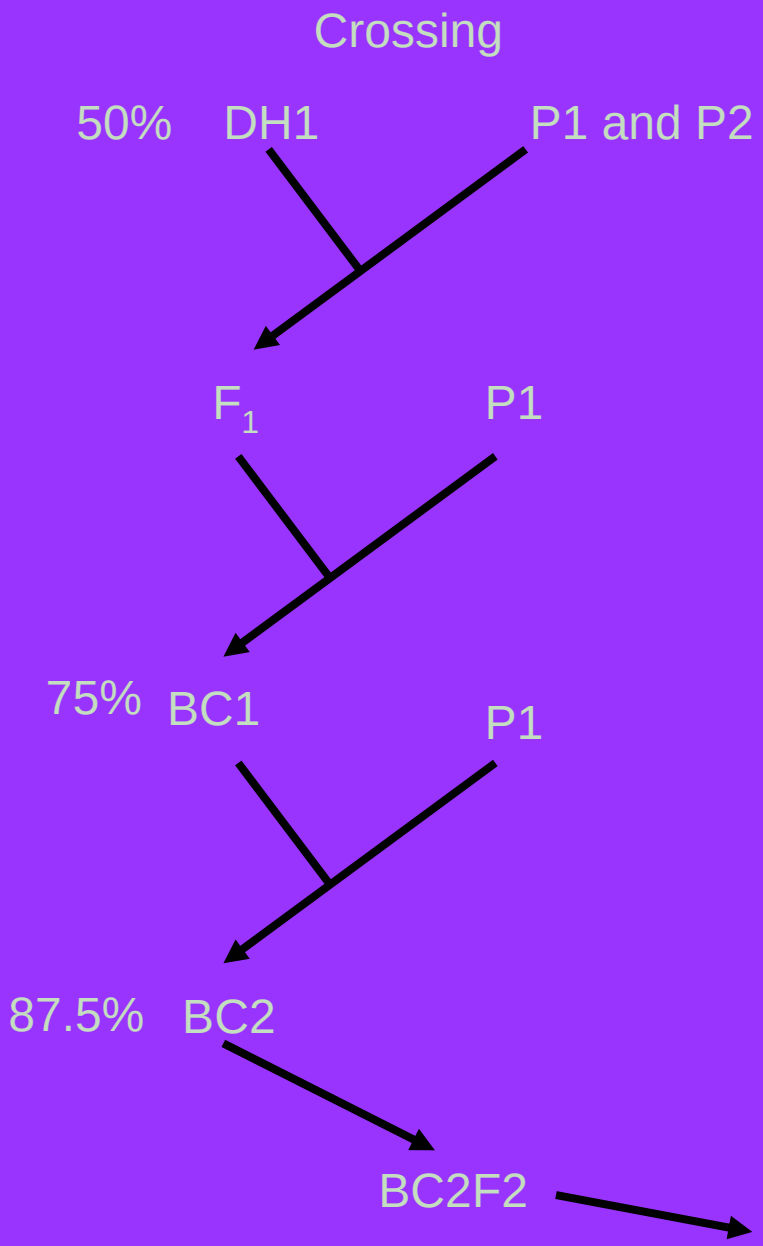
Second generation WGIN resources

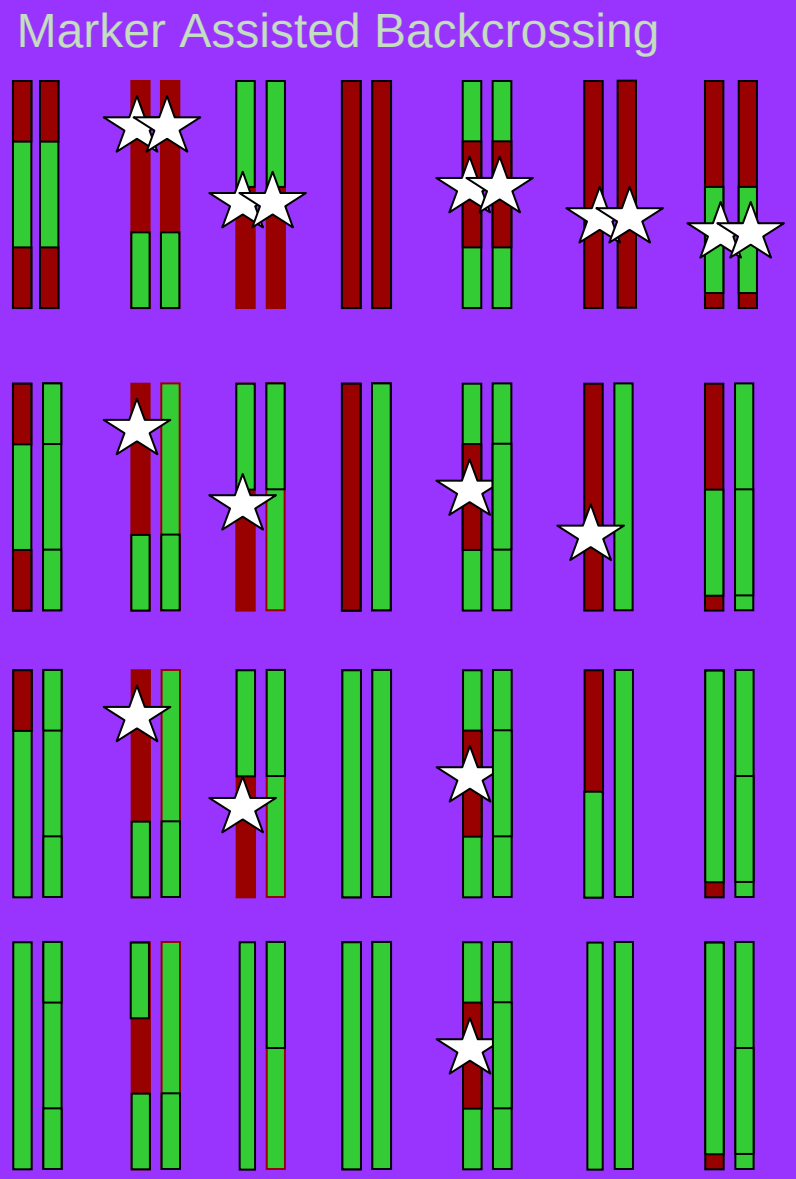
- Near Isogenic Lines
- Watkins and Gediflux DH and SSD populations
- High throughput mapping pipeline for induced mutations

Production of Near Isogenic Lines for genes of interest to WGIN

- Backcrossing underway for height, yield, and ear emergence.
- Mapping parents and Paragon used as recurrent parent.
- Complements series of NILs being produced in Paragon in other projects eg Crop Science Initiative for Rhts, Ppds, Vrns, and WGIN mutants.

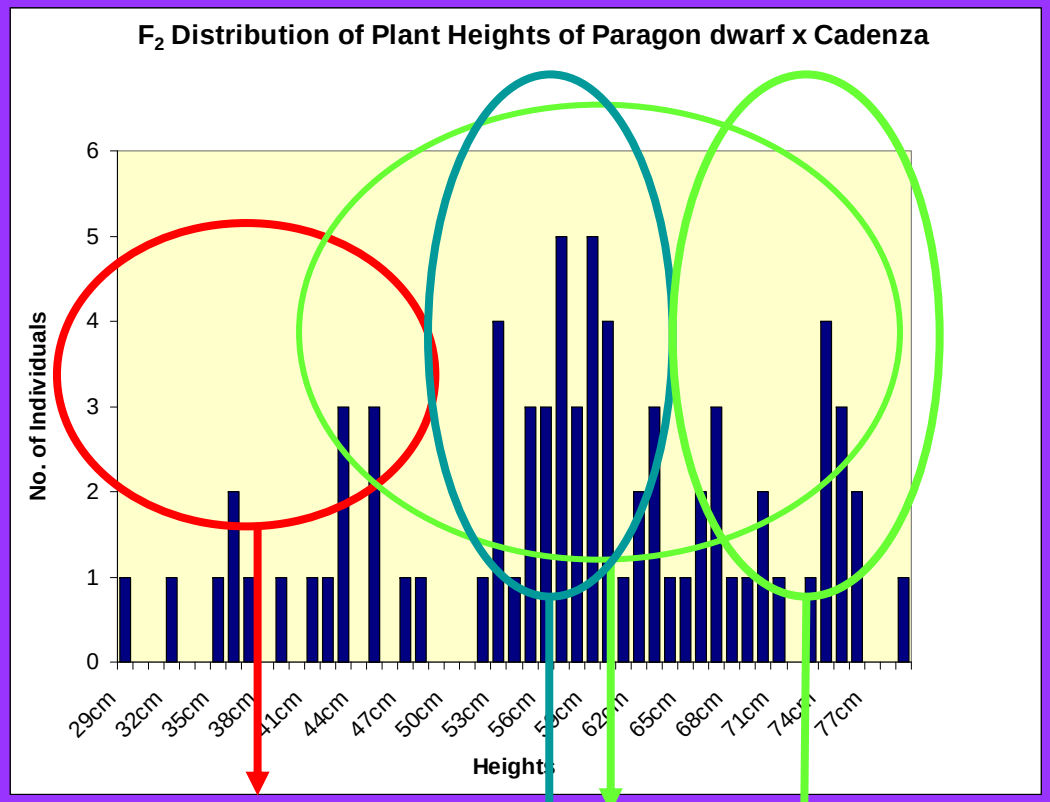
Production of Near Isogenic Lines (NILs)





Population of 32 representing both homozygous haplotypes of both parental classes

Mapping Paragon mutants in F_2s using DArT BSA



18

58

35

23

X^2=0.07

X^2=1.13

Summary

- Key WGIN resources at JIC are Avalon X Cadenza map and data, Gediflux, AE Watkins, Paragon mutants, and gene based markers

- We can chip away at monolithic germplasm resources with smart germplasm development and new genomics tools!

WGIN team

- Simon Orford — Paragon mutants and Watkins
- Michelle Leverington-Waite — COS
- Yingkun Wang — AXC mapping
- Liz Sayers — AXC phenotyping
- Leodie Alibert — AXC mapping, functional markers
- Mike Ambrose — Germplasm

- Setting it all up:
  Robert Koebner, John Snape, Pauline Stephenson, Peter Shewry, Kim Hammond Kosack, Andy Philips, Elke Anzinger

  and the UK wheat community for turning up


SPILT GRAIN IS
ENCOURAGING RATS AND
MICE

John Innes Centre

Update on germplasm and marker development at JIC

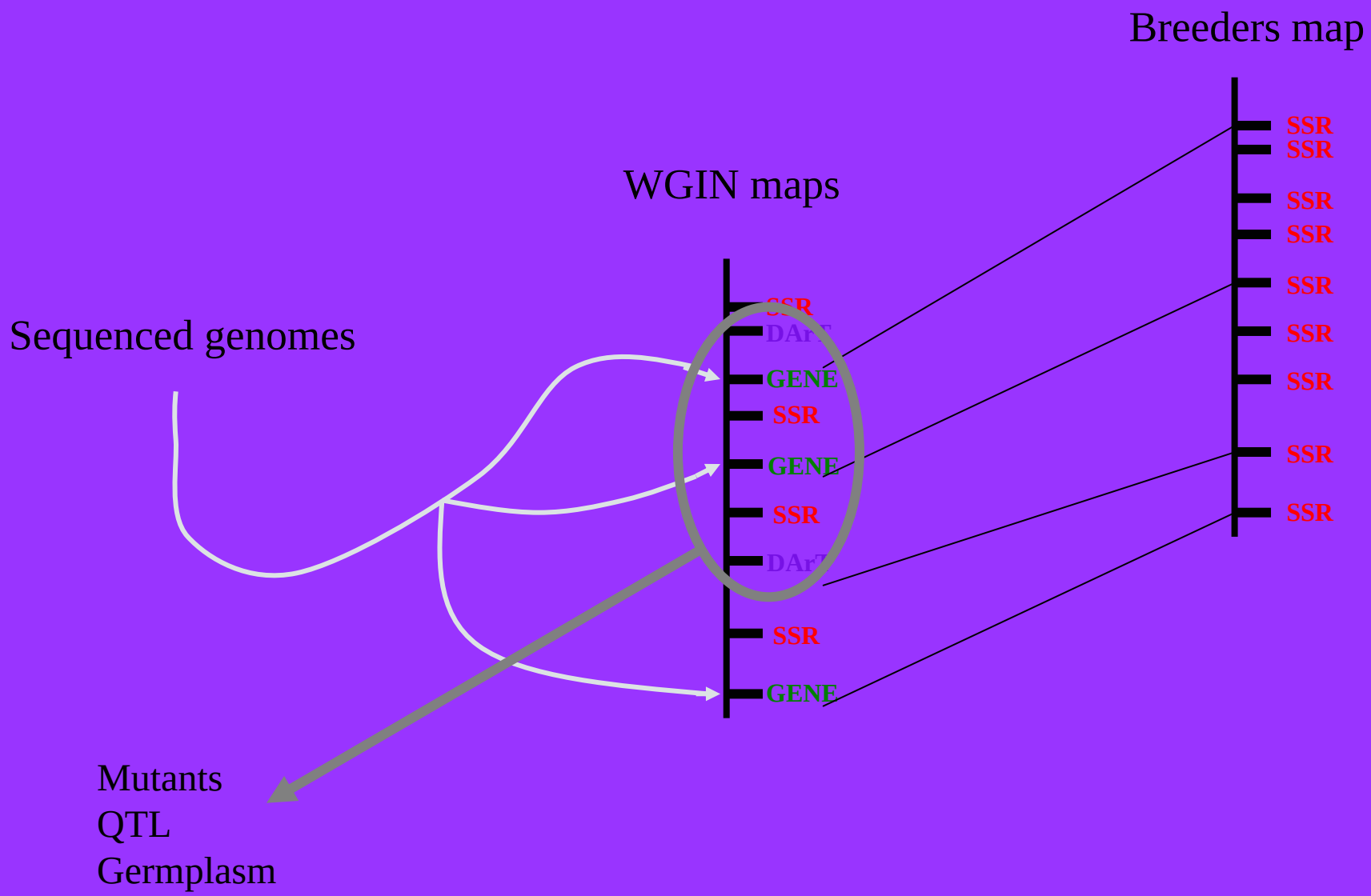
WGIN management group meeting 27th June 2007

Simon Griffiths

Contribution of JIC to WGIN

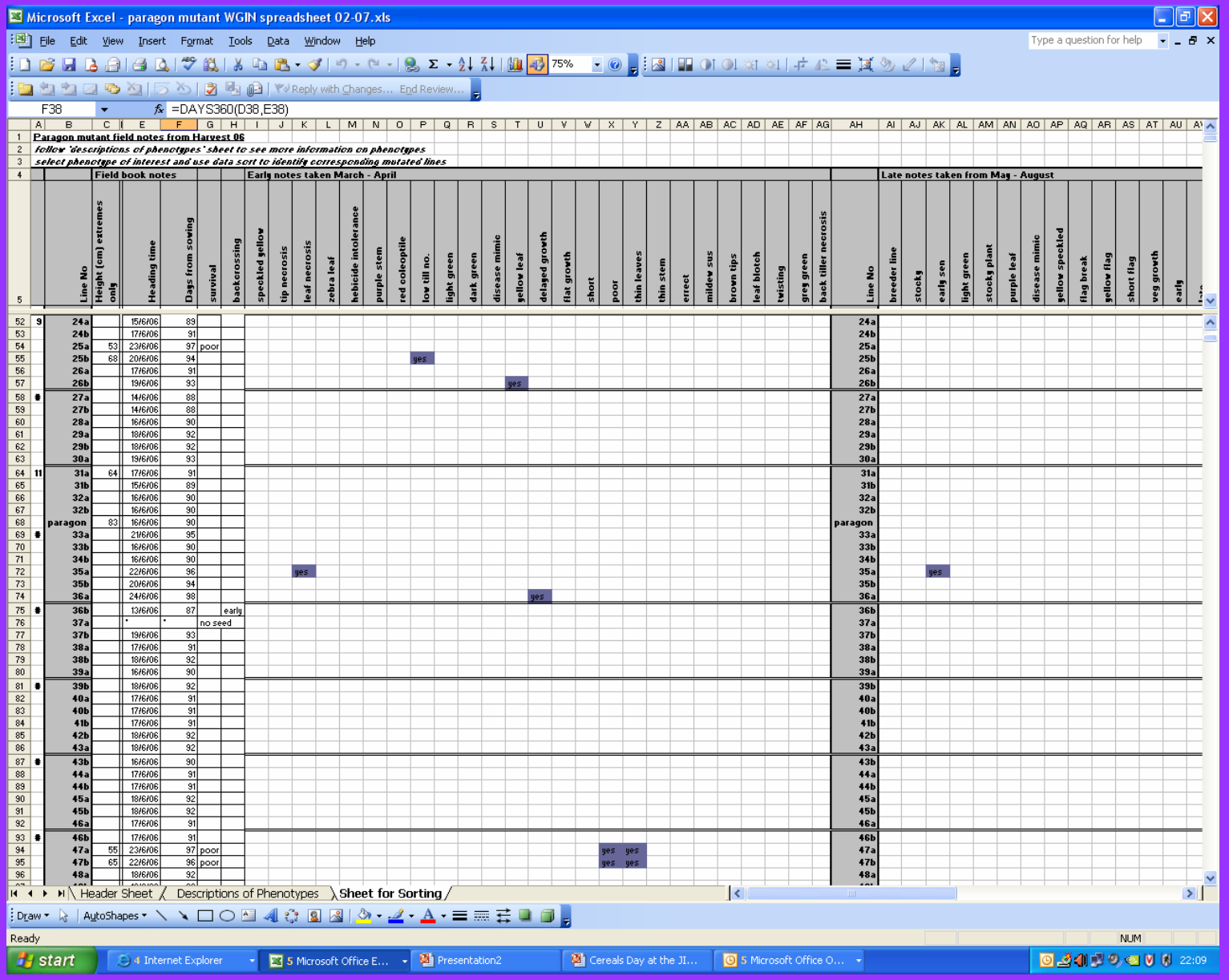
- EMS and Gamma mutated Paragon population - 6500 and 2000 lines respectively
- A. E. Watkins collection - 814 lines
- Avalon x Cadenza reference mapping population - 202 doubled haploid lines
- EU GEDIFLUX wheat variety collection from 1940s to 2000 - 510 lines
- Development of gene based markers

WGIN RESOURCES ARE WELL INTEGRATED



Paragon Mutants

Data now on ww.wgin.org.uk



Establishing a forward genetics pipeline for Paragon EMS population

- Produce F_2 using parents that are sufficiently polymorphic and will not segregate for the trait of interest.
- Good phenotyping
- Mapping using bulk segregant analysis coupled to a high throughput marker platform
- Test cases- height, disease mimic, wax, ear phenotypes

Paragon disease mimic

Wax

Gamma Paragon Mutants

- Nicola Hart Phd - Larger deletions
- Initial tests with gamma at a local Hospital
- Irradiated at IAEA Austria 25-250 Grays
- Developed to M_3 generation – further development planned

Gamma for reverse genetics

- *Ph*1 (new deletions in press)
- *Ppd-D*1, *-A*1, *-B*1
- *Rht-D*1
- *Waxy-D*1

Getting the resources used!

- Web page updates (Elke)
- JIC field walks
- Cereals 2007
- EWAC Meeting (Istanbul)
- Friends of John Innes
- JIC Breeders day

CEREALS 2007

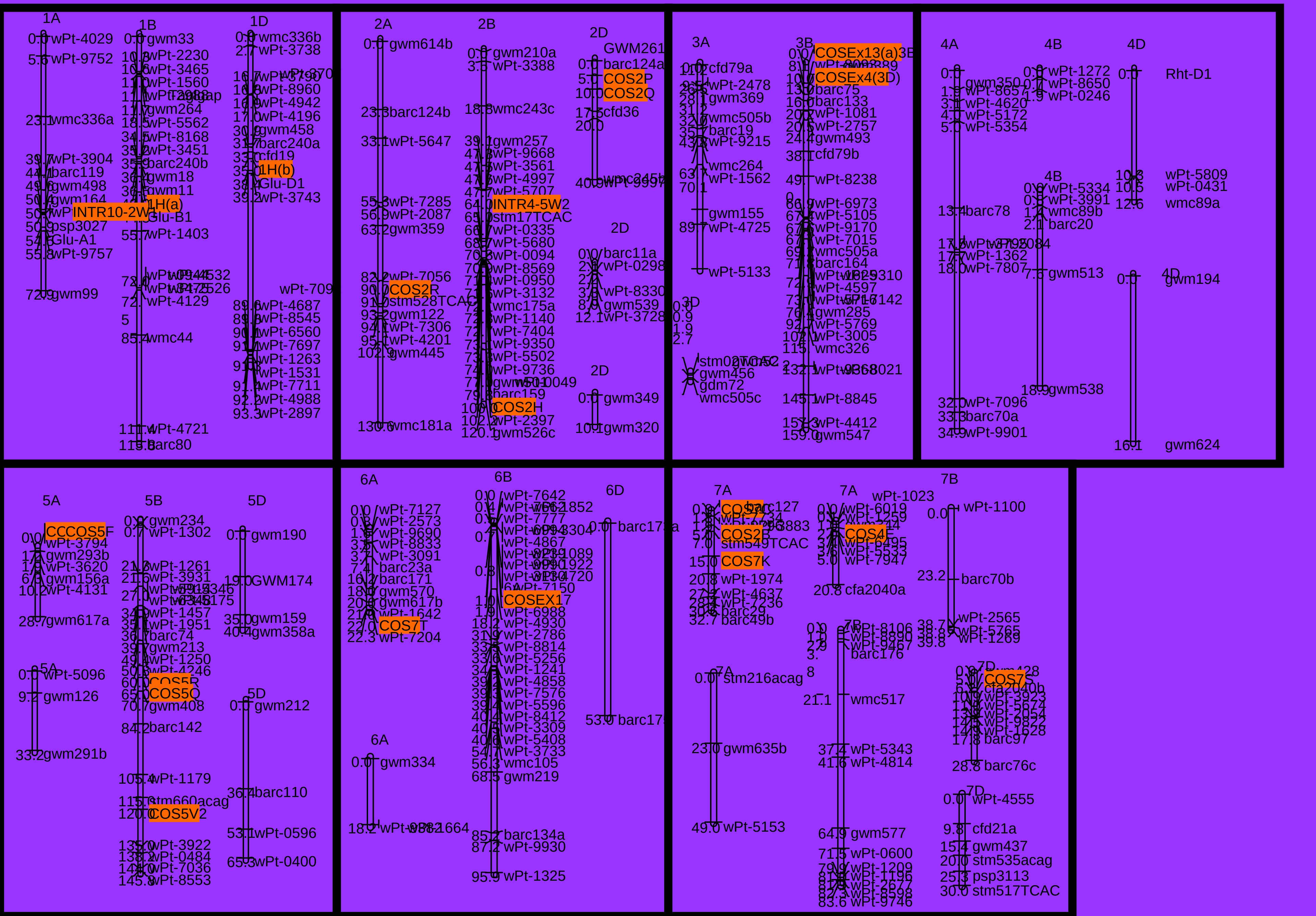
Good uptake of Paragon material

- CIMMYT X 2
- Adelaide
- 2 breeding companies
- ADAS
- NIAB

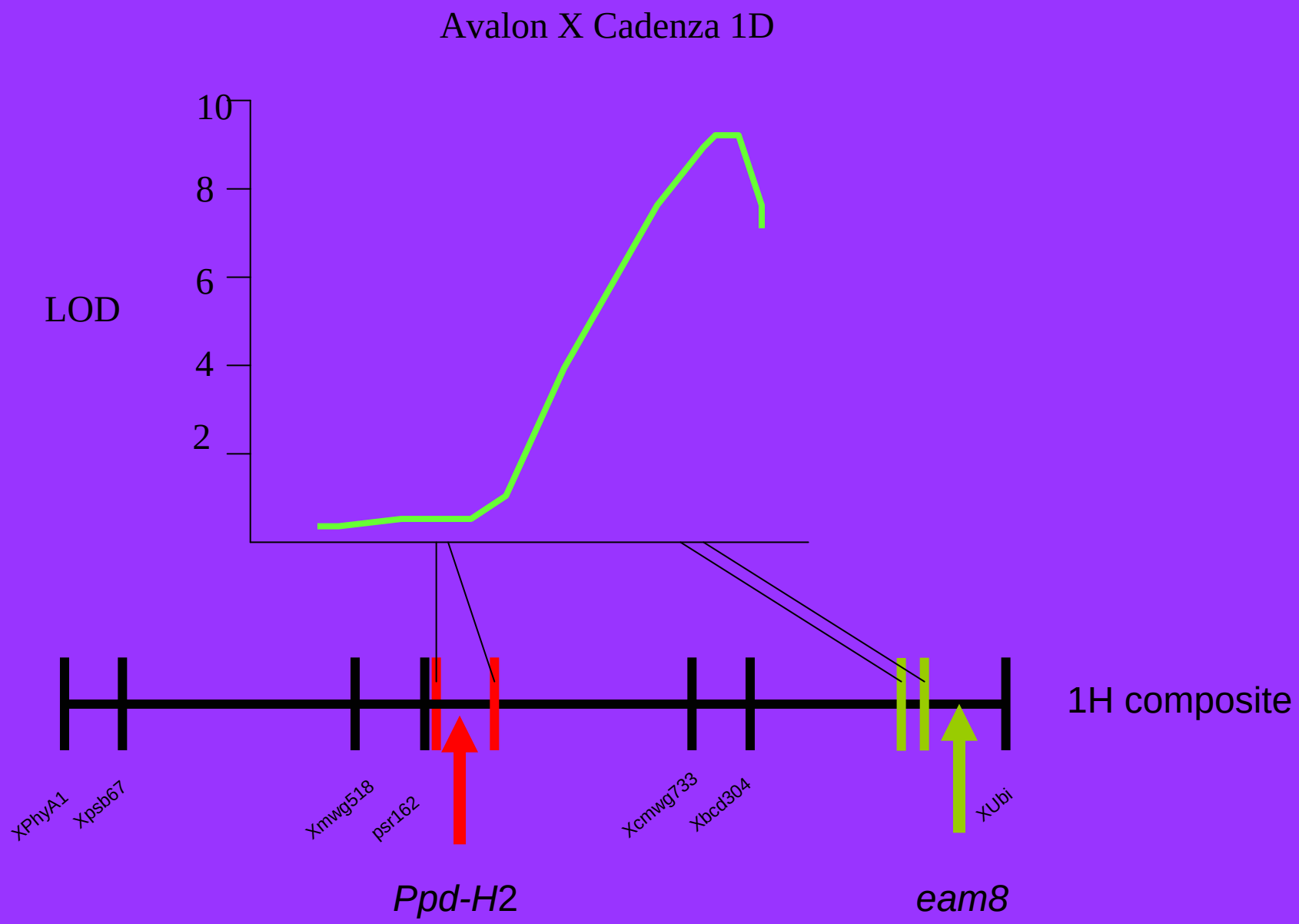
- Material transferred under JIC public collections MTA

Gene based markers (COS)

Gene based markers on Avalon X Cadenza map



Alignment of wheat and barley flowering time effects; 1D v 1H



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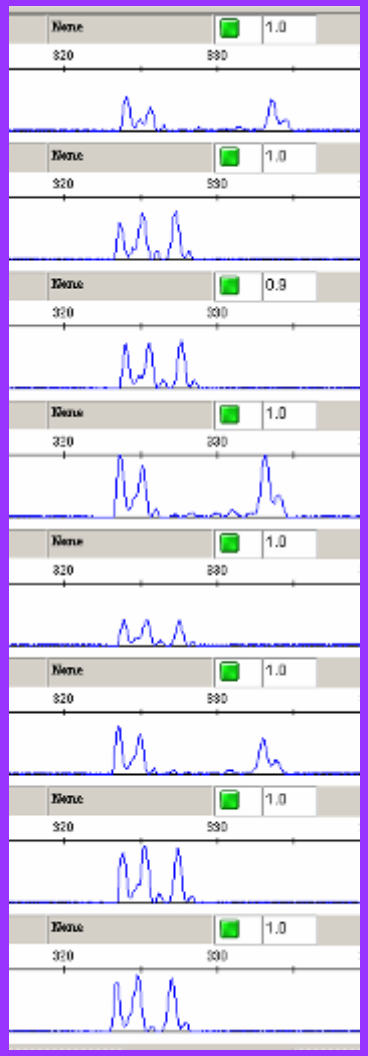
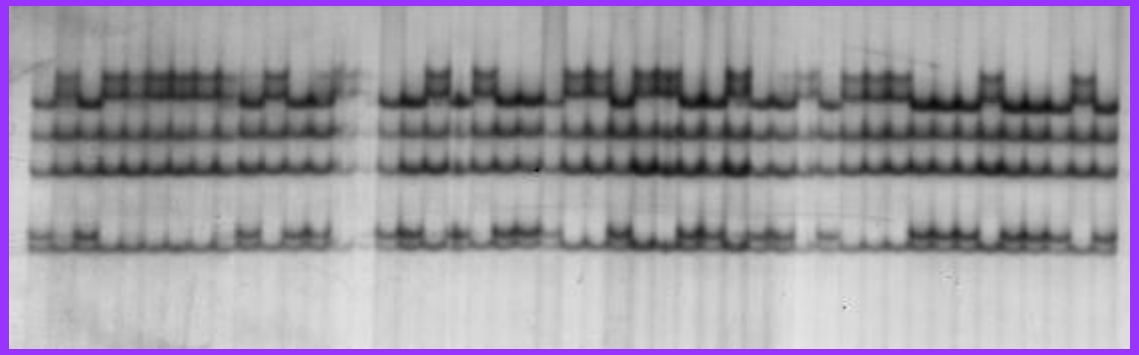
Single Strand Conformation Polymorphism is now a high throughput technique





Conventional SSCP ran on non-denaturing acrylamide

ABI3730 capillary electrophoresis (modified polymer, CAP3)

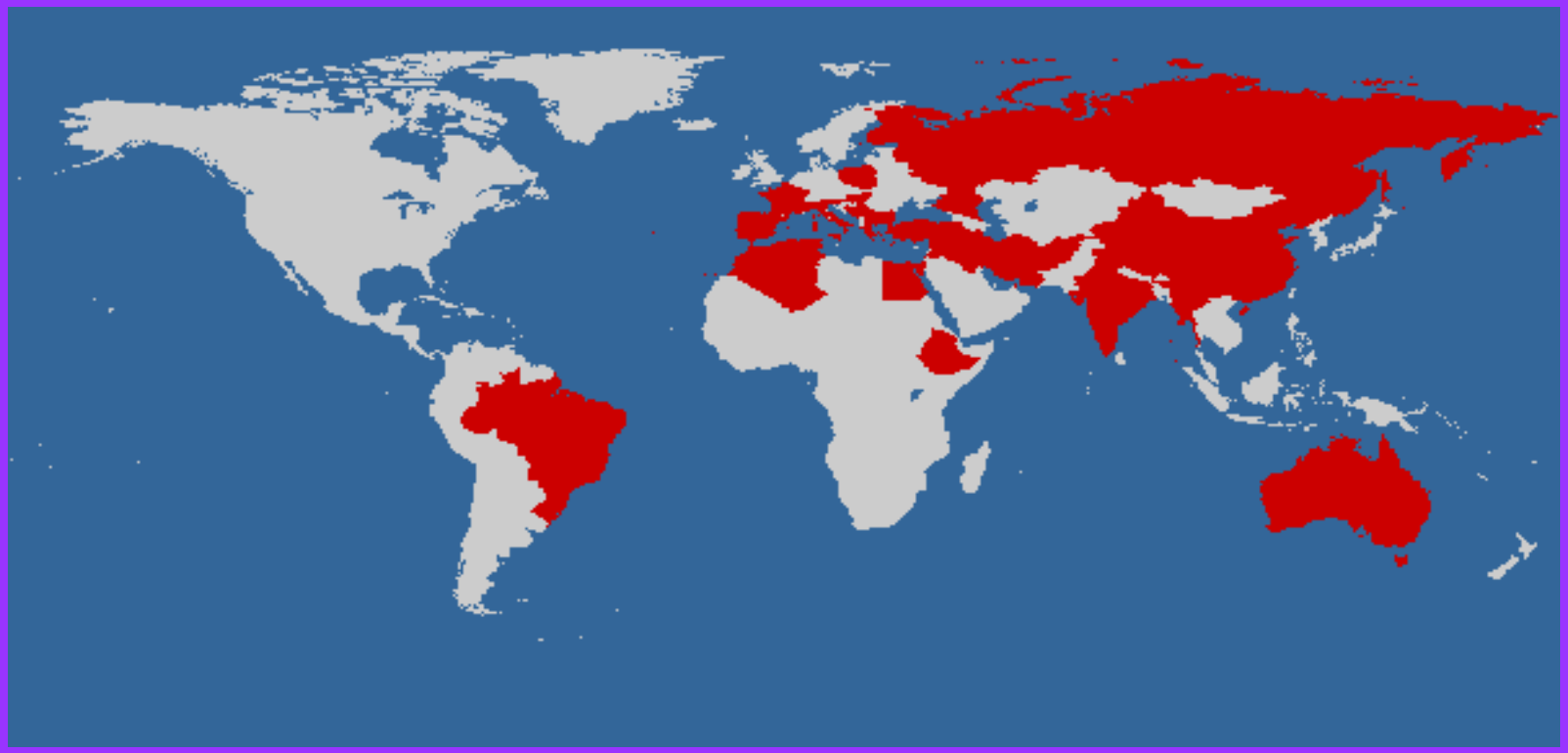
Data now on www.wgin.org.uk

Levels of polymorphism detected in COS markers: SSCP gels cf. fluorescent detection on ABI

Wheat Group	Number of Markers designed	Amplifies in Wheat	Poly AxC on ABI	Poly AxC on SSCP gels	Poly ITMI on ABI	Poly ITMI on SSCP gels
1	19	19	2	1 out of 14 tried	3	1 out of 6 tried
2	17	17	4	6 out of 13 tried	7	0 out of 8 tried
3	17	17	5	0 out of 12 tried	5	1 out of 6 tried
4	21	21	7	5 out of 14 tried	7	2 out of 4 tried
5	21	18	6	6 out of 15 tried	11	2 out of 6 tried
6	16	15	3	2 out of 10 tried	5	1 out of 5 tried
7	13	13	2	4 out of 11 tried	5	1 out of 5 tried
Total	124	120	29	24/89	43	8/40

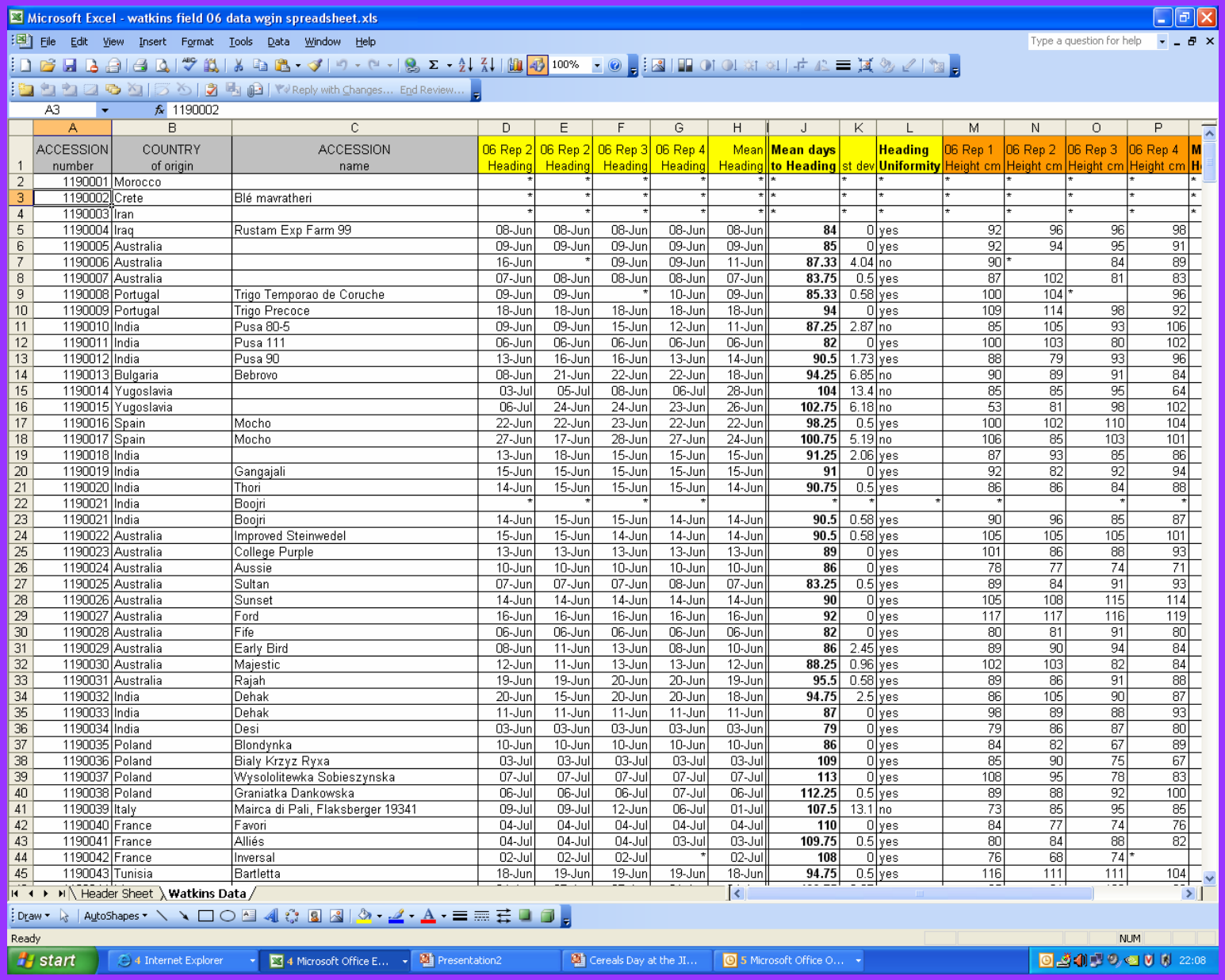
Watkins Collection

Origins of Watkins Collection



- Africa: Algeria, Canary Islands, Egypt, Ethiopia, Morocco, Tunisia,
- Asia and Middle East: Afghanistan, Burma, China, India, Iran, Iraq, Palestine, Syria, Turkey,
- Europe: Bulgaria, Crete, Cyprus, Finland, France, Greece, Hungary, Italy, Poland, Portugal, Romania, Spain, UK, USSR, Yugoslavia,
- Australia and Brazil

Data now on www.wgin.org.uk



Microsoft Excel - watkins field 06 data wgin spreadsheet.xls

	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P
1	ACCESSION number	COUNTRY of origin	ACCESSION name	06 Rep 2 Heading	06 Rep 2 Heading	06 Rep 3 Heading	06 Rep 4 Heading	Mean Heading	Mean days to Heading	st dev	Heading Uniformity	06 Rep 1 Height cm	06 Rep 2 Height cm	06 Rep 3 Height cm	06 Rep 4 Height cm
2	1190001	Morocco		*	*	*	*	*	*	*	*	*	*	*	*
3	1190002	Crete	Blé mavratheri	*	*	*	*	*	*	*	*	*	*	*	*
4	1190003	Iran		*	*	*	*	*	*	*	*	*	*	*	*
5	1190004	Iraq	Rustam Exp Farm 99	08-Jun	08-Jun	08-Jun	08-Jun	08-Jun	**84**	0	yes	92	96	96	98
6	1190005	Australia		09-Jun	09-Jun	09-Jun	09-Jun	09-Jun	**85**	0	yes	92	94	95	91
7	1190006	Australia		16-Jun	*	09-Jun	09-Jun	11-Jun	**87.33**	4.04	no	90	*	84	89
8	1190007	Australia		07-Jun	08-Jun	08-Jun	08-Jun	07-Jun	**83.75**	0.5	yes	87	102	81	83
9	1190008	Portugal	Trigo Temporao de Coruche	09-Jun	09-Jun	*	10-Jun	09-Jun	**85.33**	0.58	yes	100	104	*	96
10	1190009	Portugal	Trigo Precoce	18-Jun	18-Jun	18-Jun	18-Jun	18-Jun	**94**	0	yes	109	114	98	92
11	1190010	India	Pusa 80-5	09-Jun	09-Jun	15-Jun	12-Jun	11-Jun	**87.25**	2.87	no	85	105	93	106
12	1190011	India	Pusa 111	06-Jun	06-Jun	06-Jun	06-Jun	06-Jun	**82**	0	yes	100	103	80	102
13	1190012	India	Pusa 90	13-Jun	16-Jun	16-Jun	13-Jun	14-Jun	**90.5**	1.73	yes	88	79	93	96
14	1190013	Bulgaria	Bebrovo	08-Jun	21-Jun	22-Jun	22-Jun	18-Jun	**94.25**	6.85	no	90	89	91	84
15	1190014	Yugoslavia		03-Jul	05-Jul	08-Jun	06-Jul	28-Jun	**104**	13.4	no	85	85	95	64
16	1190015	Yugoslavia		06-Jul	24-Jun	24-Jun	23-Jun	26-Jun	**102.75**	6.18	no	53	81	98	102
17	1190016	Spain	Mocho	22-Jun	22-Jun	23-Jun	22-Jun	22-Jun	**98.25**	0.5	yes	100	102	110	104
18	1190017	Spain	Mocho	27-Jun	17-Jun	28-Jun	27-Jun	24-Jun	**100.75**	5.19	no	106	85	103	101
19	1190018	India		13-Jun	18-Jun	15-Jun	15-Jun	15-Jun	**91.25**	2.06	yes	87	93	85	86
20	1190019	India	Gangajali	15-Jun	15-Jun	15-Jun	15-Jun	15-Jun	**91**	0	yes	92	82	92	94
21	1190020	India	Thori	14-Jun	15-Jun	15-Jun	15-Jun	14-Jun	**90.75**	0.5	yes	86	86	84	88
22	1190021	India	Boojri	*	*	*	*		*	*	*	*		*	*
23	1190021	India	Boojri	14-Jun	15-Jun	15-Jun	14-Jun	14-Jun	**90.5**	0.58	yes	90	96	85	87
24	1190022	Australia	Improved Steinwedel	15-Jun	15-Jun	14-Jun	14-Jun	14-Jun	**90.5**	0.58	yes	105	105	105	101
25	1190023	Australia	College Purple	13-Jun	13-Jun	13-Jun	13-Jun	13-Jun	**89**	0	yes	101	86	88	93
26	1190024	Australia	Aussie	10-Jun	10-Jun	10-Jun	10-Jun	10-Jun	**86**	0	yes	78	77	74	71
27	1190025	Australia	Sultan	07-Jun	07-Jun	07-Jun	08-Jun	07-Jun	**83.25**	0.5	yes	89	84	91	93
28	1190026	Australia	Sunset	14-Jun	14-Jun	14-Jun	14-Jun	14-Jun	**90**	0	yes	105	108	115	114
29	1190027	Australia	Ford	16-Jun	16-Jun	16-Jun	16-Jun	16-Jun	**92**	0	yes	117	117	116	119
30	1190028	Australia	Fife	06-Jun	06-Jun	06-Jun	06-Jun	06-Jun	**82**	0	yes	80	81	91	80
31	1190029	Australia	Early Bird	08-Jun	11-Jun	13-Jun	08-Jun	10-Jun	**86**	2.45	yes	89	90	94	84
32	1190030	Australia	Majestic	12-Jun	11-Jun	13-Jun	13-Jun	12-Jun	**88.25**	0.96	yes	102	103	82	84
33	1190031	Australia	Rajah	19-Jun	19-Jun	20-Jun	20-Jun	19-Jun	**95.5**	0.58	yes	89	86	91	88
34	1190032	India	Dehak	20-Jun	15-Jun	20-Jun	20-Jun	18-Jun	**94.75**	2.5	yes	86	105	90	87
35	1190033	India	Dehak	11-Jun	11-Jun	11-Jun	11-Jun	11-Jun	**87**	0	yes	98	89	88	93
36	1190034	India	Desi	03-Jun	03-Jun	03-Jun	03-Jun	03-Jun	**79**	0	yes	79	86	87	80
37	1190035	Poland	Blondynka	10-Jun	10-Jun	10-Jun	10-Jun	10-Jun	**86**	0	yes	84	82	67	89
38	1190036	Poland	Bialy Krzyz Ryxa	03-Jul	03-Jul	03-Jul	03-Jul	03-Jul	**109**	0	yes	85	90	75	67
39	1190037	Poland	Wysololitewka Sobieszynska	07-Jul	07-Jul	07-Jul	07-Jul	07-Jul	**113**	0	yes	108	95	78	83
40	1190038	Poland	Graniatka Dankowska	06-Jul	06-Jul	06-Jul	07-Jul	06-Jul	**112.25**	0.5	yes	89	88	92	100
41	1190039	Italy	Mairca di Pali, Flaksberger 19341	09-Jul	09-Jul	12-Jun	06-Jul	01-Jul	**107.5**	13.1	no	73	85	95	85
42	1190040	France	Favori	04-Jul	04-Jul	04-Jul	04-Jul	04-Jul	**110**	0	yes	84	77	74	76
43	1190041	France	Alliés	04-Jul	04-Jul	04-Jul	03-Jul	03-Jul	**109.75**	0.5	yes	80	84	88	82
44	1190042	France	Inversal	02-Jul	02-Jul	02-Jul	*	02-Jul	**108**	0	yes	76	68	74	*
45	1190043	Tunisia	Bartletta	18-Jun	19-Jun	19-Jun	19-Jun	18-Jun	**94.75**	0.5	yes	116	111	111	104

Header Sheet | Watkins Data

Ready

NUM

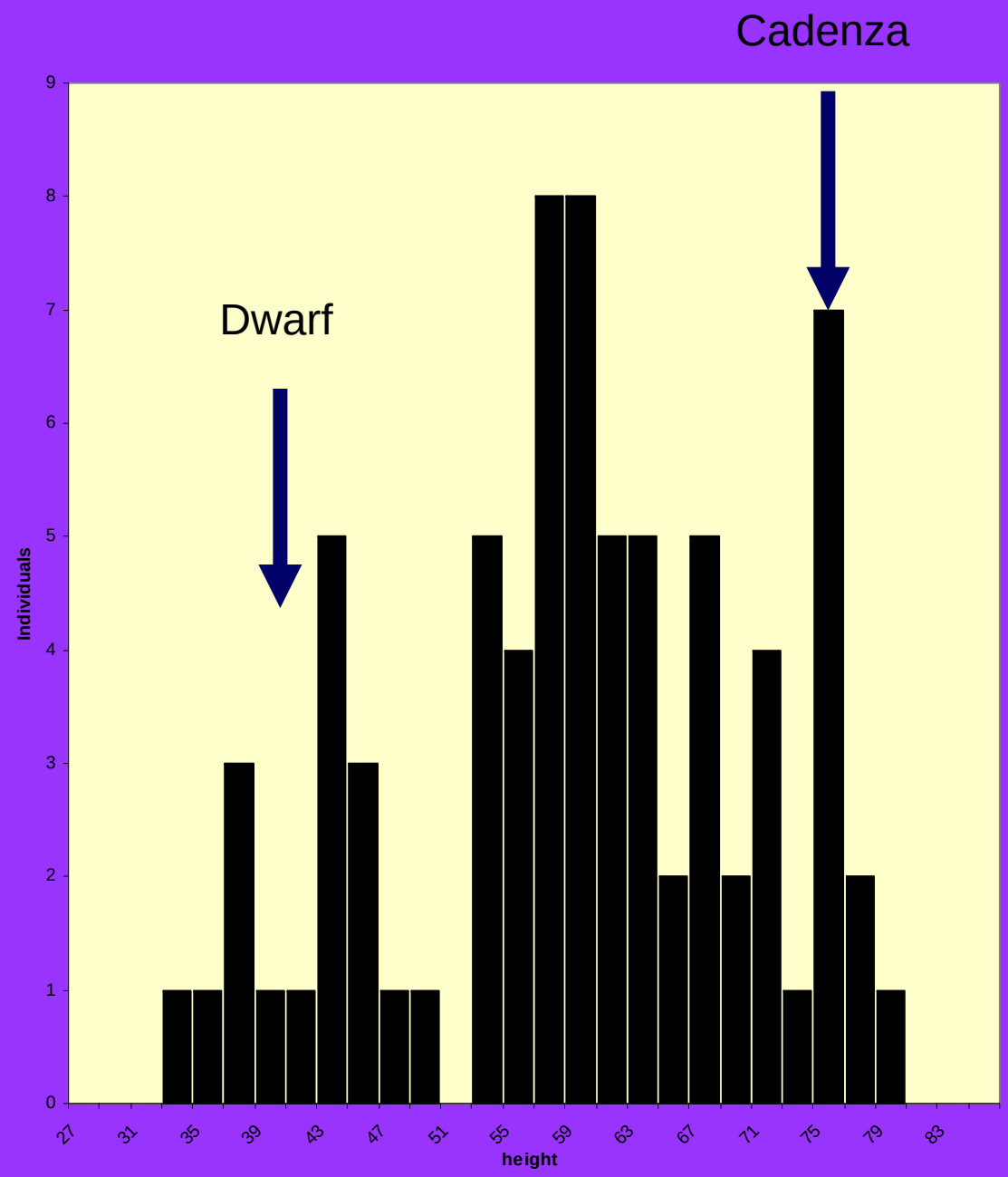
start | 4 Internet Explorer | 4 Microsoft Office E... | Presentation2 | Cereals Day at the JI... | 5 Microsoft Office O... | 22:08

How to strengthen these resources

- Watkins needs deeper genotyping- whole genome and known function markers
- More gamma and/or fast neutron irradiated material needed
- Gediflux is a great resource- add spring varieties especially Paragon and more markers.

WGIN team at JIC

• John Snape	Particular interest in AXC
• Simon Orford	Paragon mutants and Watkins
• Michelle Leverington-Waite	COS
• Yingkun Wang	AXC mapping
• Liz Sayers	AXC phenotyping
• Leodie Alibert	AXC mapping, functional markers
• Mike Ambrose	Germplasm


Cadenza
Dwarf
Individuals
height
0
1
2
3
4
5
6
7
8
9
27
31
35
39
43
47
51
55
59
63
67
71
75
79
83

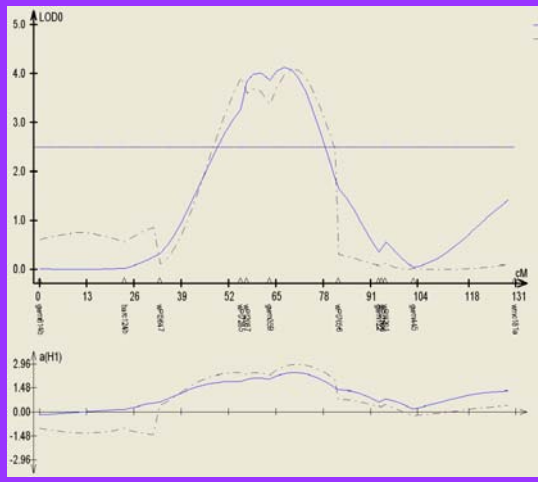
Discovering what genetic variation UK wheat breeders are using- height

- Variation in final plant height is the result of a wide range of developmental effects of direct relevance to yield, yield stability, lodging, biomass, and resource use efficiency.

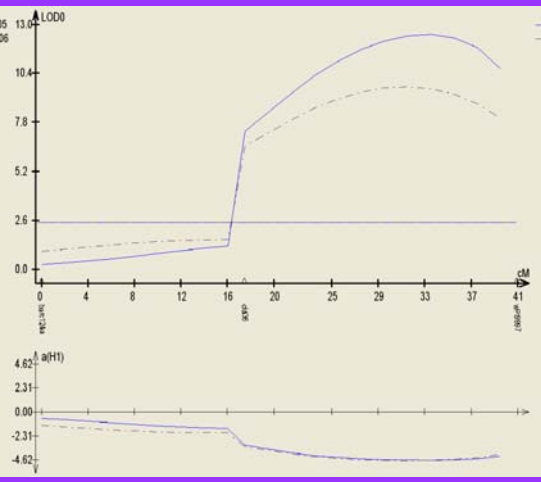
- What genes do wheat breeders use to manipulate final plant height?

Genes controlling plant height in Avalon X Cadenza population

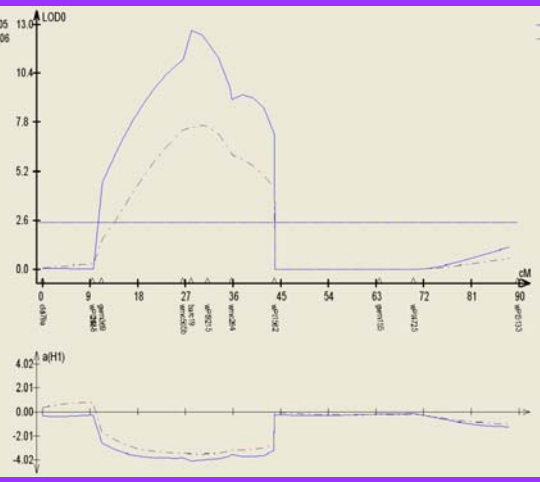
2A



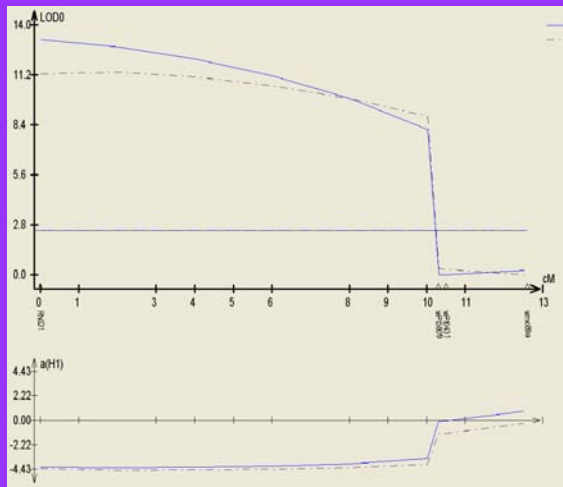
2D



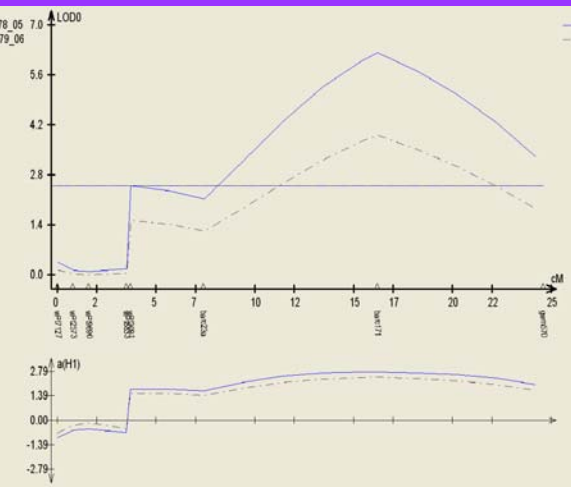
3A



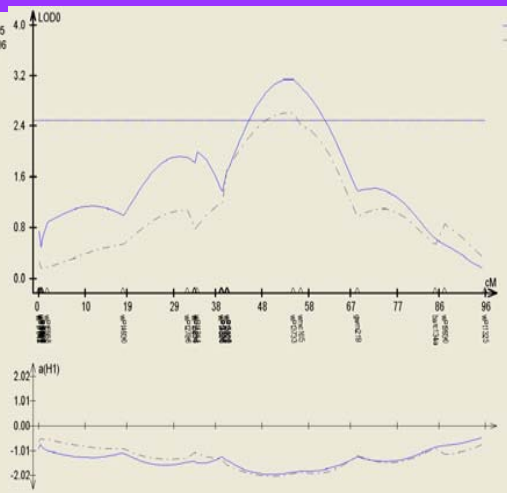
4D



6A



6B



Overview of WGIN Resources

- **Germplasm**

  Avalon x Cadenza, EMS and gamma Paragon mutants, Cadenza mutant lines, AE Watkins Collection, Gediflux Collection, precise genetic stocks, *T. monococcum* material.

- **Tools**

  SSCP marker development, DArT gamma deletion index, Tilling-Melt curve platform, DArT-BSA mutant locus mapping.

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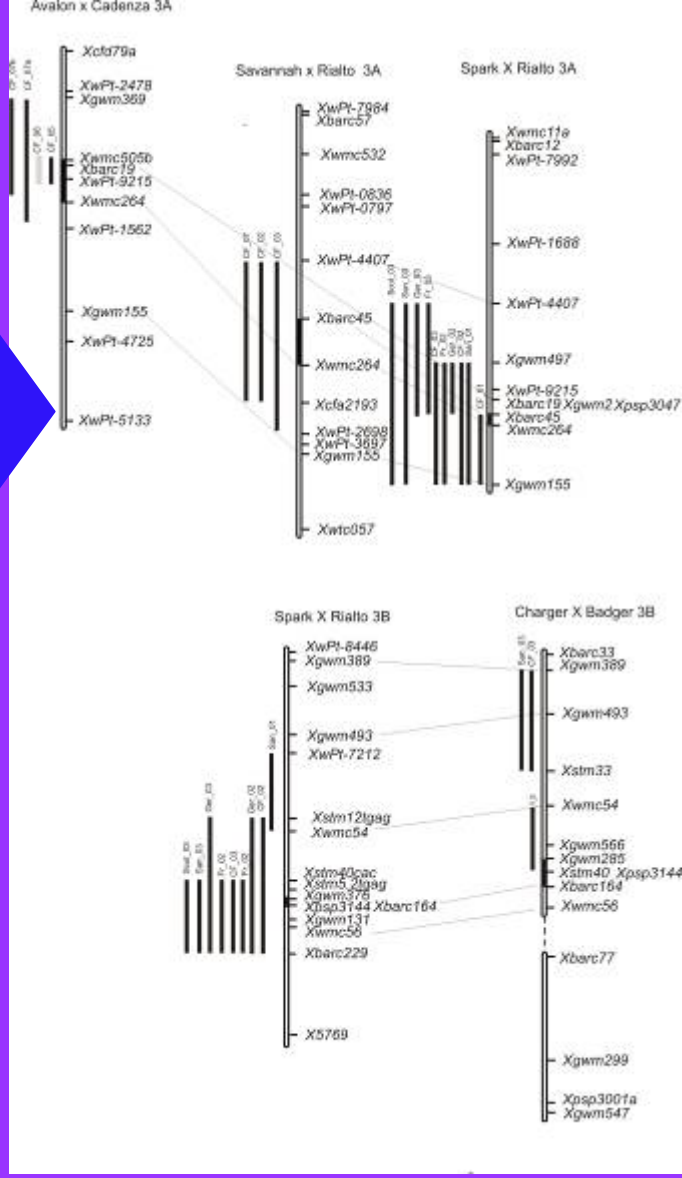
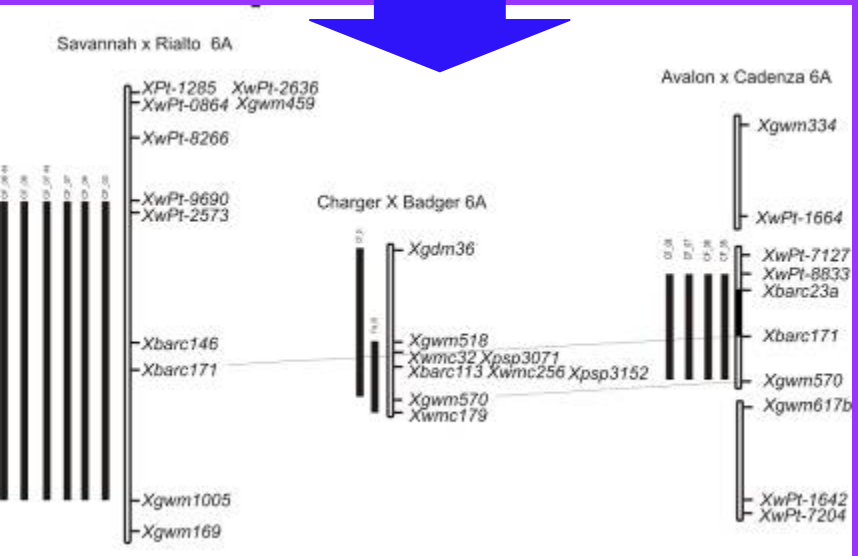
Avalon Cadenza ear emergence and height QTL in comparison to other UK adapted crosses

Avalon x Cadenza
Spark x Rialto
Savannah x Rialto
Charger x Badger

EAR EMERGENCE

HEIGHT





Avalon x Cadenza Mapping Population

- **Avalon**
- **Parents: Maris Ploughman x Bilbo**
- **-Winter wheat**
- **Pinb-D1b allele present**
- **5B-7B translocation**
- **1/6+8/2+12 HMW proteins**
- **Rht-D1 dwarf**

- **Cadenza**
- **Parents: Axona x Tonic**
- **Spring type wheat**
- **Pinb-D1c allele present**
- **Normal 5B, 7B**
- **N/14+15/5+10 HMW proteins**
- **no dwarfing genes**