



The James
Hutton
Institute

La recombinaison chez les plantes : contrôle par les facteurs du milieu

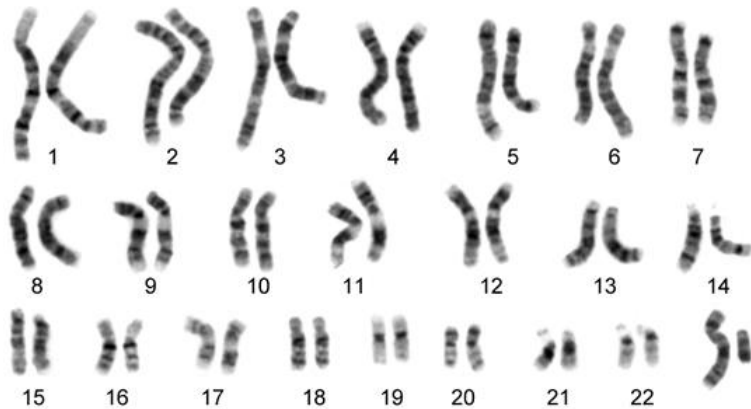
Luke Ramsay

Barley (orge)

- Barley
- Inbreeding diploid cereal
- Large genome (large chromosomes)
- Important to Scotland
- Breeding programmes across Europe (Limagrain, Syngenta, Secobra, RAGT, KWS, etc.)



Human : Barley comparison



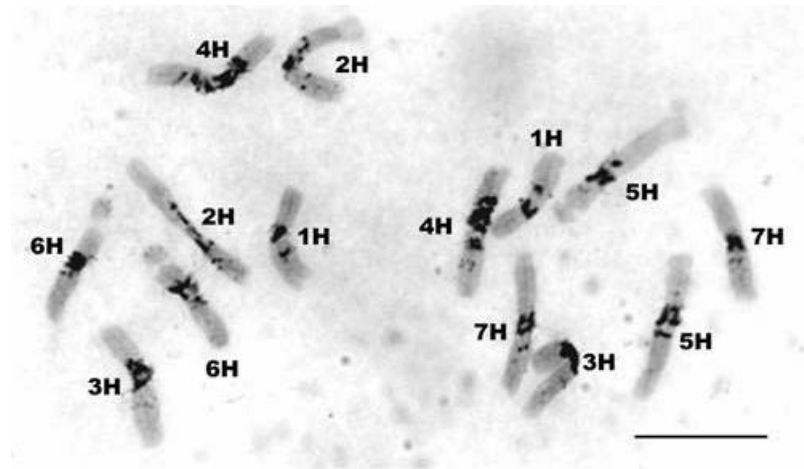
Human

20,000-25,000 genes

3,200,000,000 bp

23 chromosome pairs

Ave 69.8 Crossovers/cell



Barley

26,159 high-confidence genes

5,100,000,000 bp

7 chromosome pairs

21.8 Crossovers/cell

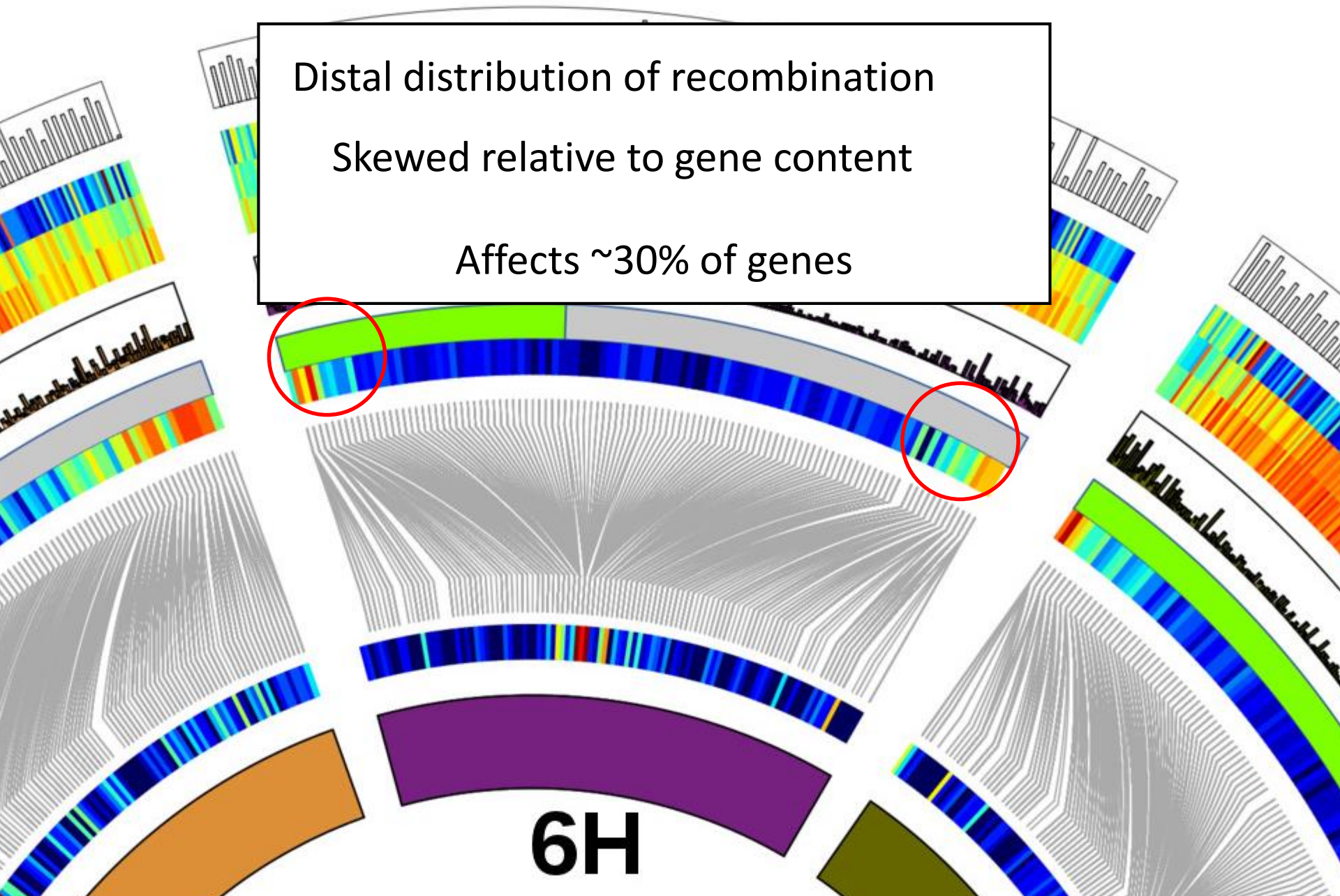
Effect of independent assortment weaker and less recombination

Problem of distribution of recombination as well !

Distal distribution of recombination

Skewed relative to gene content

Affects ~30% of genes



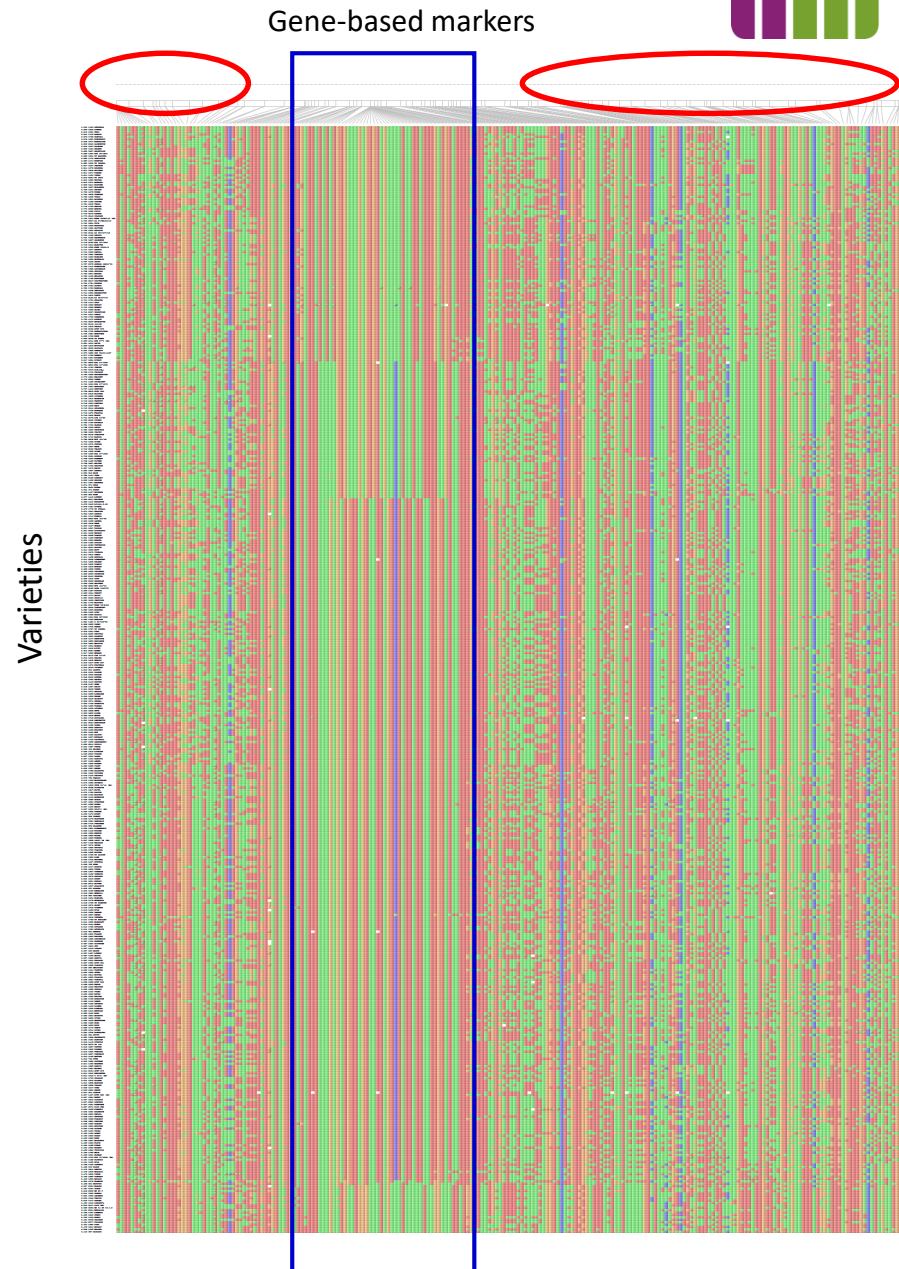
Patterns of Diversity in UK barleys

Most recombination has been
between genes at the ends of
the chromosome

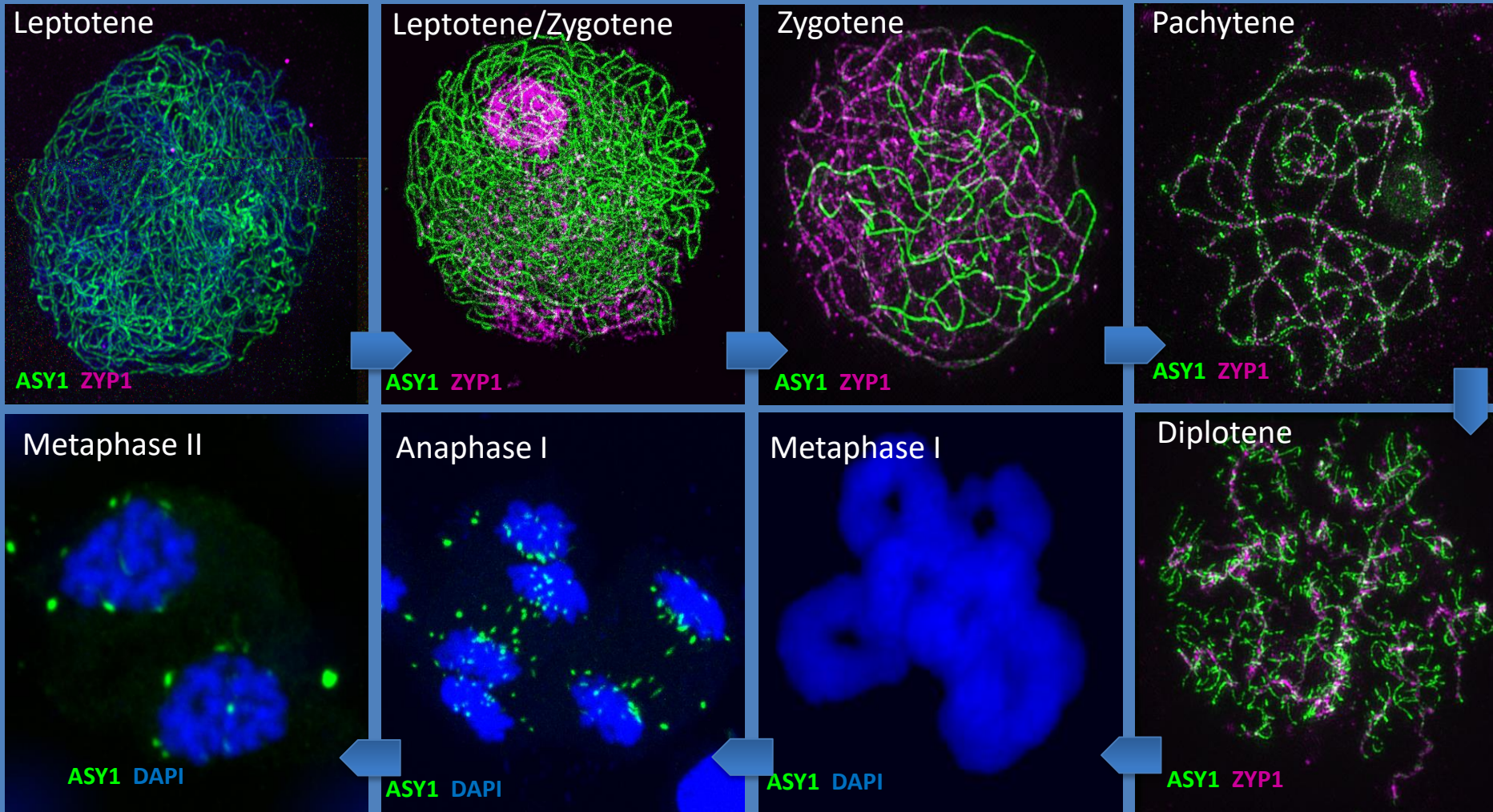
Limited recombination between
genes in rest of the chromosome

Constraint on breeding progress

Cluster of beta glucan genes 7H
Disease resistance introgression

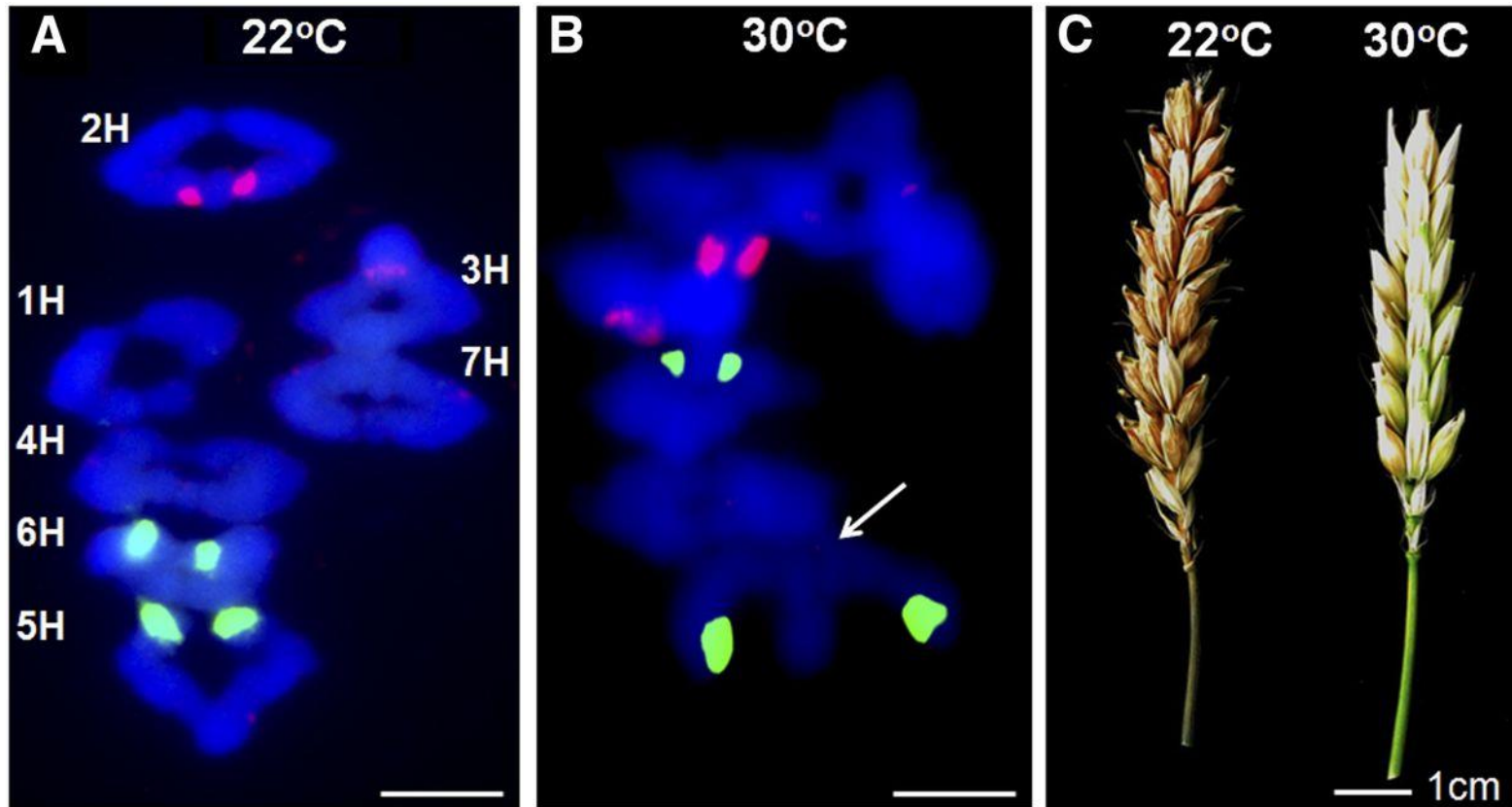


Cytological assay of barley meiosis - Isabelle Colas



Axis protein **ASY1** (ASYNAPSIS1) Synapsis **ZYP1** (ZIPPER1)

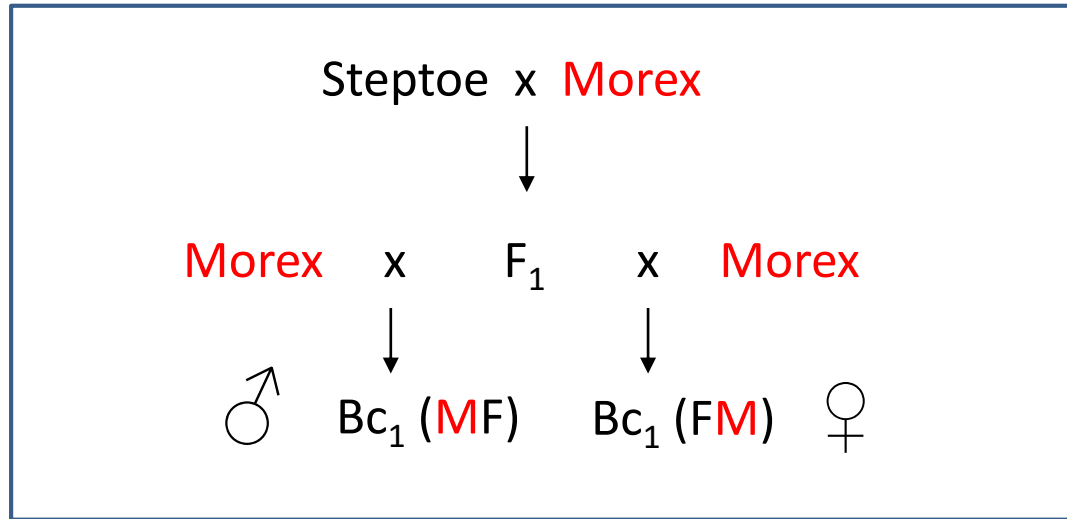
Effect of temperature: barley



Higgins et al 2012

Barley plants (cv Morex) given heat stress (30°C) show more interstitial chiasmata in Metaphase I spreads ... but fewer grains

Reciprocal backcrosses



Crosses between two inbred six-rowed varieties 'Steptoe' & 'Morex'

Crosses made with F₁ grown at 15°C, 25°C & 30°C

Six Bc₁ populations – reciprocal and temperature differences

96 Bc₁ progeny genotyped with 384 BeadXpress Veracode SNP assay

Heterochiasmy

	Male		Female	Ratio
15°C	987.1 cM		956.2 cM	1.05
25°C	1115.5 cM		920.3 cM	1.20
30°C	1359.3 cM		869.0 cM	1.58

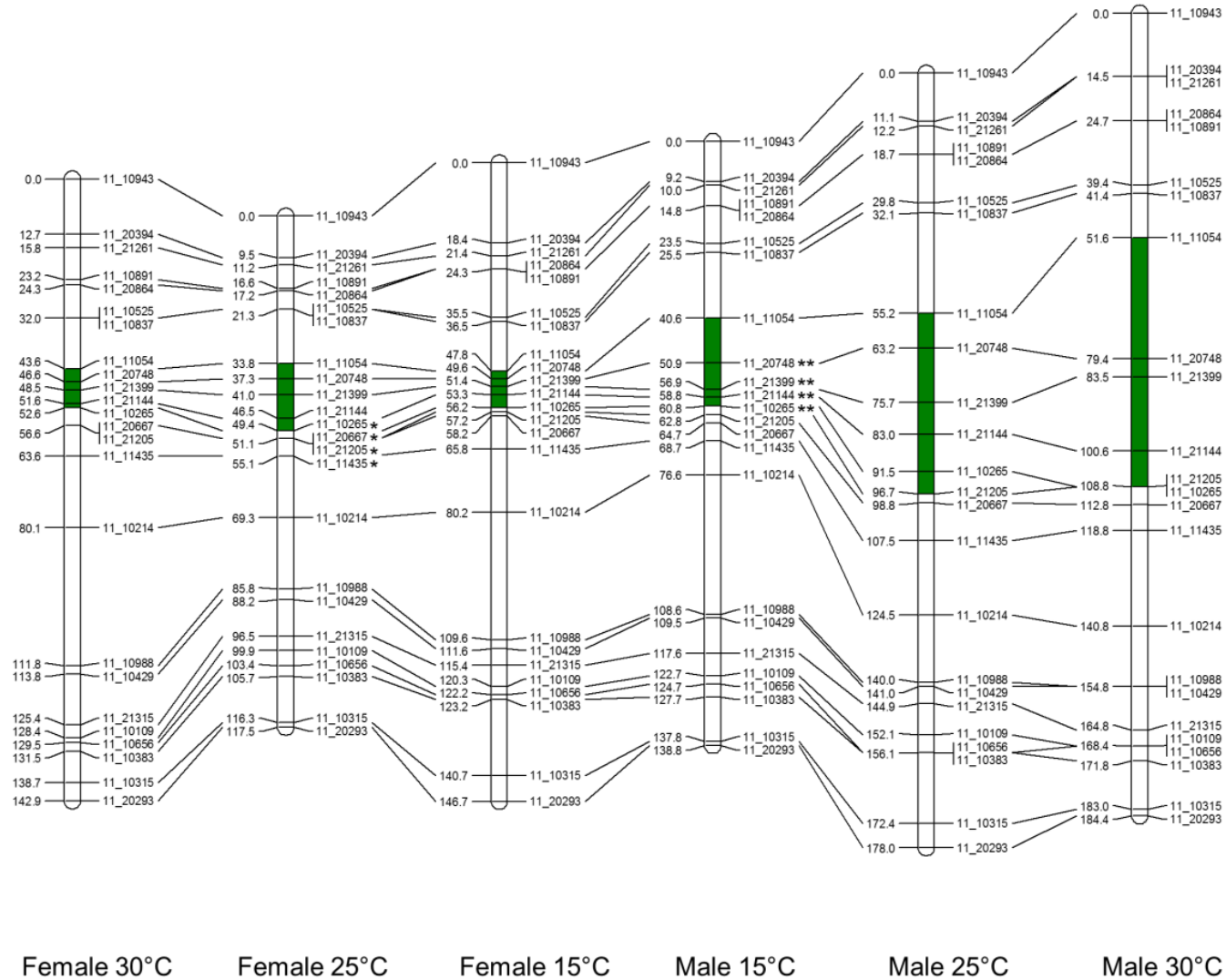
Standard consensus map of regions covered = 969.2 cM (total 1090 cM)

Increase in total map length with increasing temperature on male side

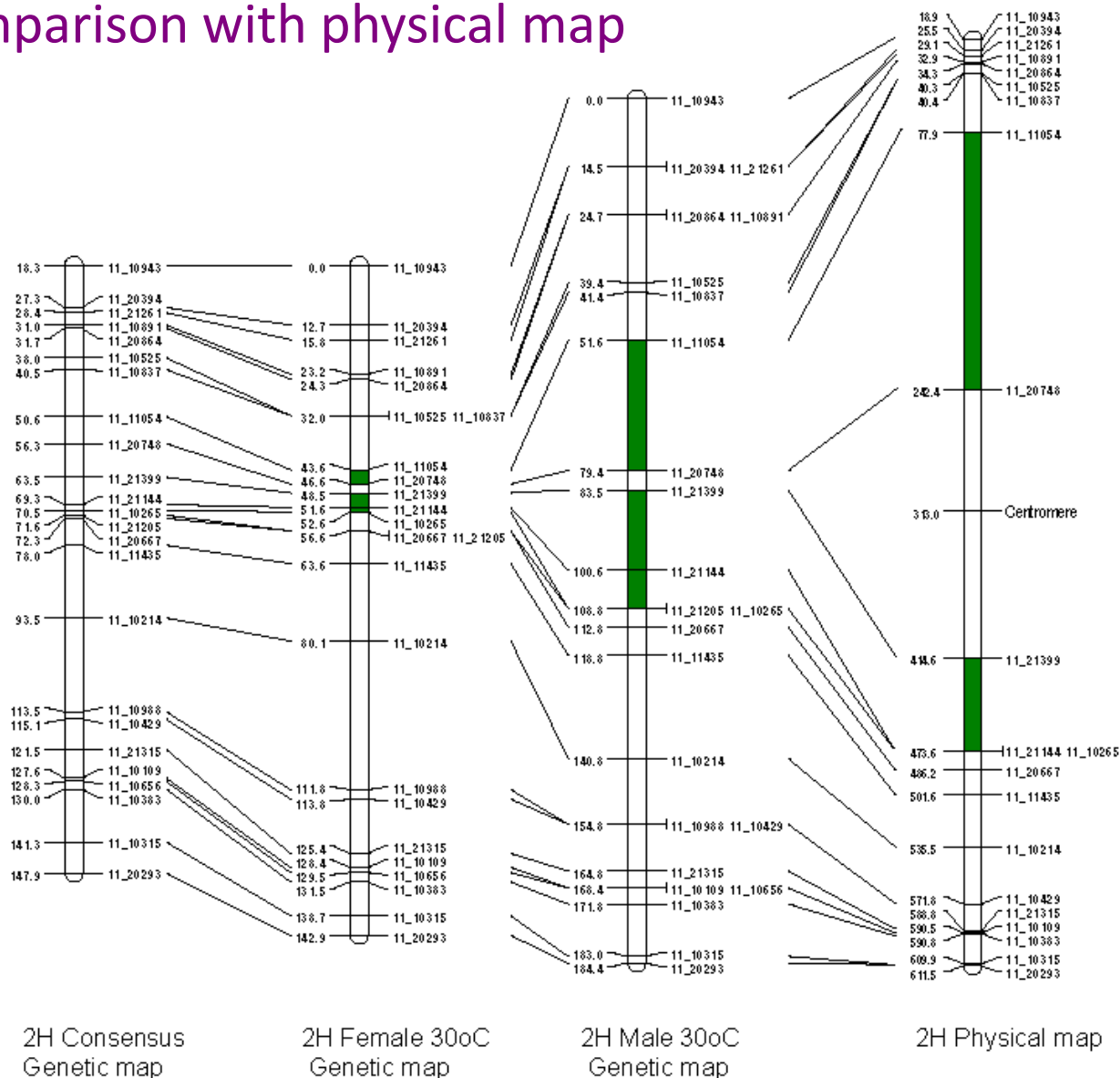
Significant male: female difference

Significant interaction with temperature

Bc₁ map comparisons – 2H

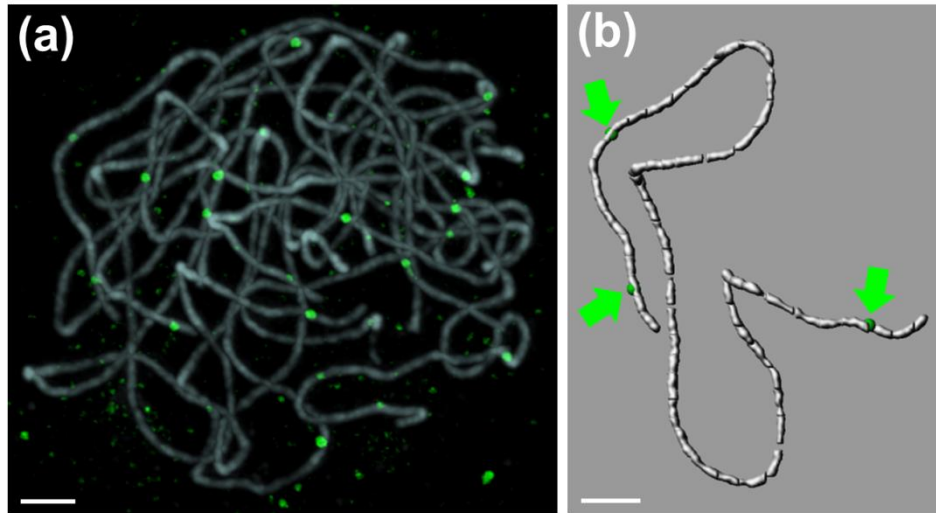


Comparison with physical map



Interstitial peri-centromeric region 9 cM – 57 cM ~400Mb

MLH3 foci mark crossover positions (Class I)



- (a) Immunolocalisation of ZYP1 (grey) and HvMLH3 (green) in an embedded pachytene nucleus.
(b) A single bivalent (grey) with the position of the HvMLH3 foci (green foci, arrowed).

MLH3 foci can be visualised at pachytene and the position and number determined

The distribution of MLH3 foci are more interstitial at 25°C than at 15 °C

Correspondence of cytological and genetic mapping estimates

Male/female asynchrony

212

M. D. BENNETT AND R. A. FINCH

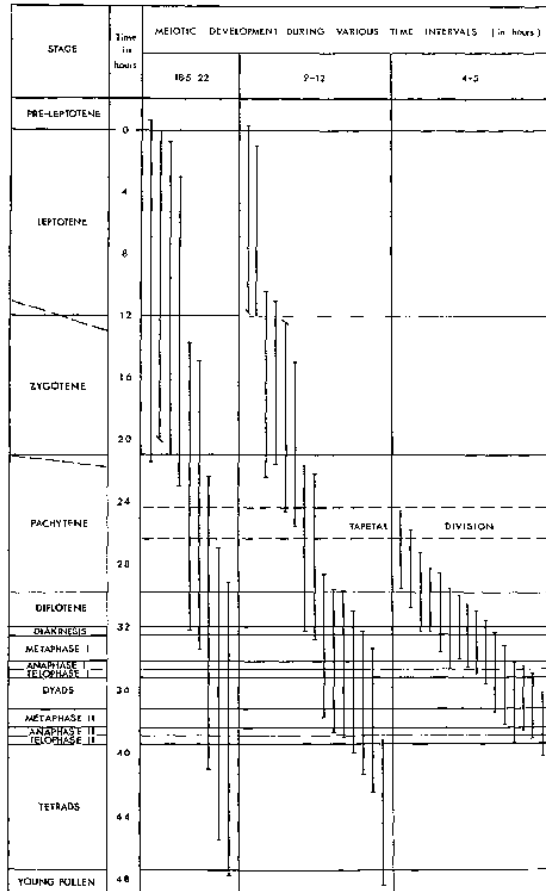


Fig. 2. Meiotic development completed during various time periods by Sultau authors at 20 °C.

Evidence from older literature that temperature stress induces asynchrony between progression of male and female meioses.

Corresponds with the shorter S phase in stressed barley plants (Higgins *et al.*, 2012)

Importance of pre-meiotic stages and entry into meiosis

Bennett & Finch 1971
Bennett *et al.*, 1973

Tool for breeding ??

Problems with long heat stress: loss of fertility (<20%)

Use of short heat stress targeting pre-meiotic/early meiosis only

Problems with accurate staging for when meiosis happens and with asynchronous flowering in barley

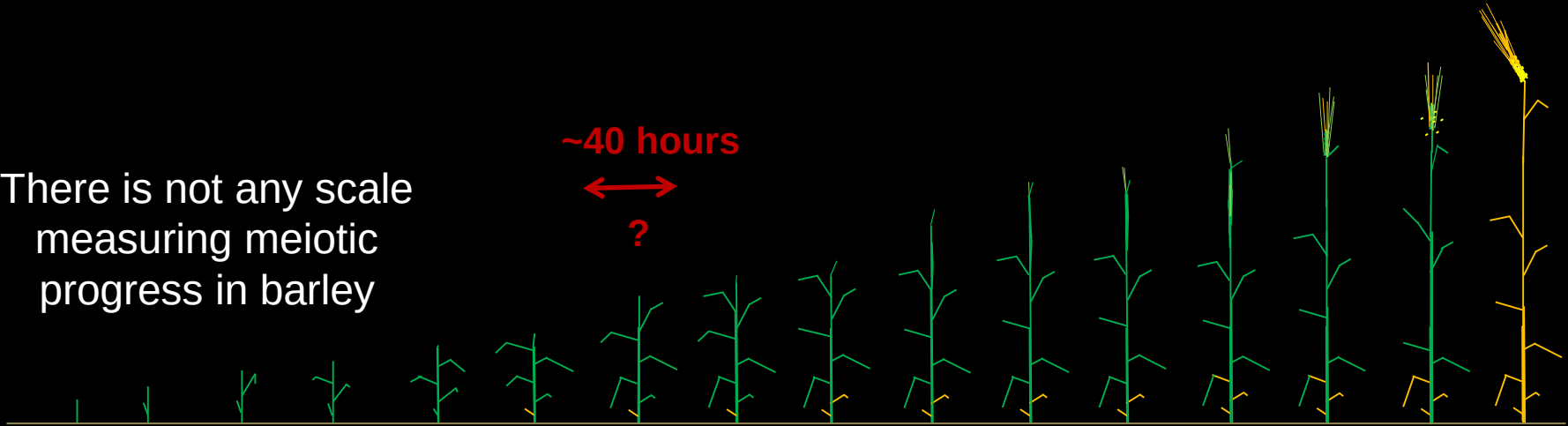
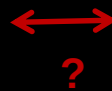




Is a targeted Heat shock possible?

There is not any scale
measuring meiotic
progress in barley

~40 hours

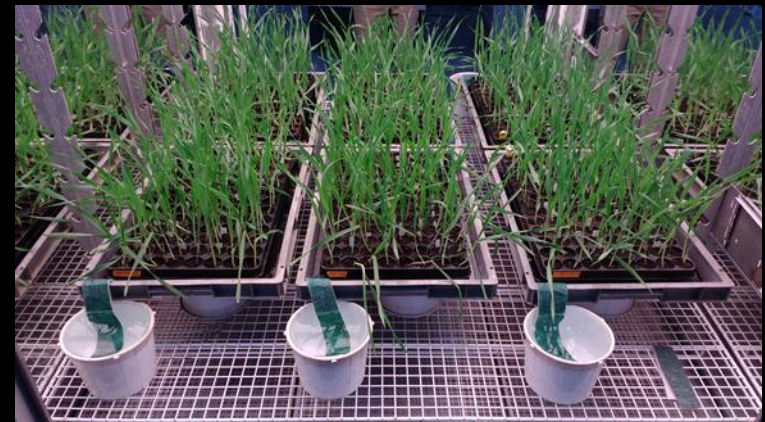


6 inch pot plantings



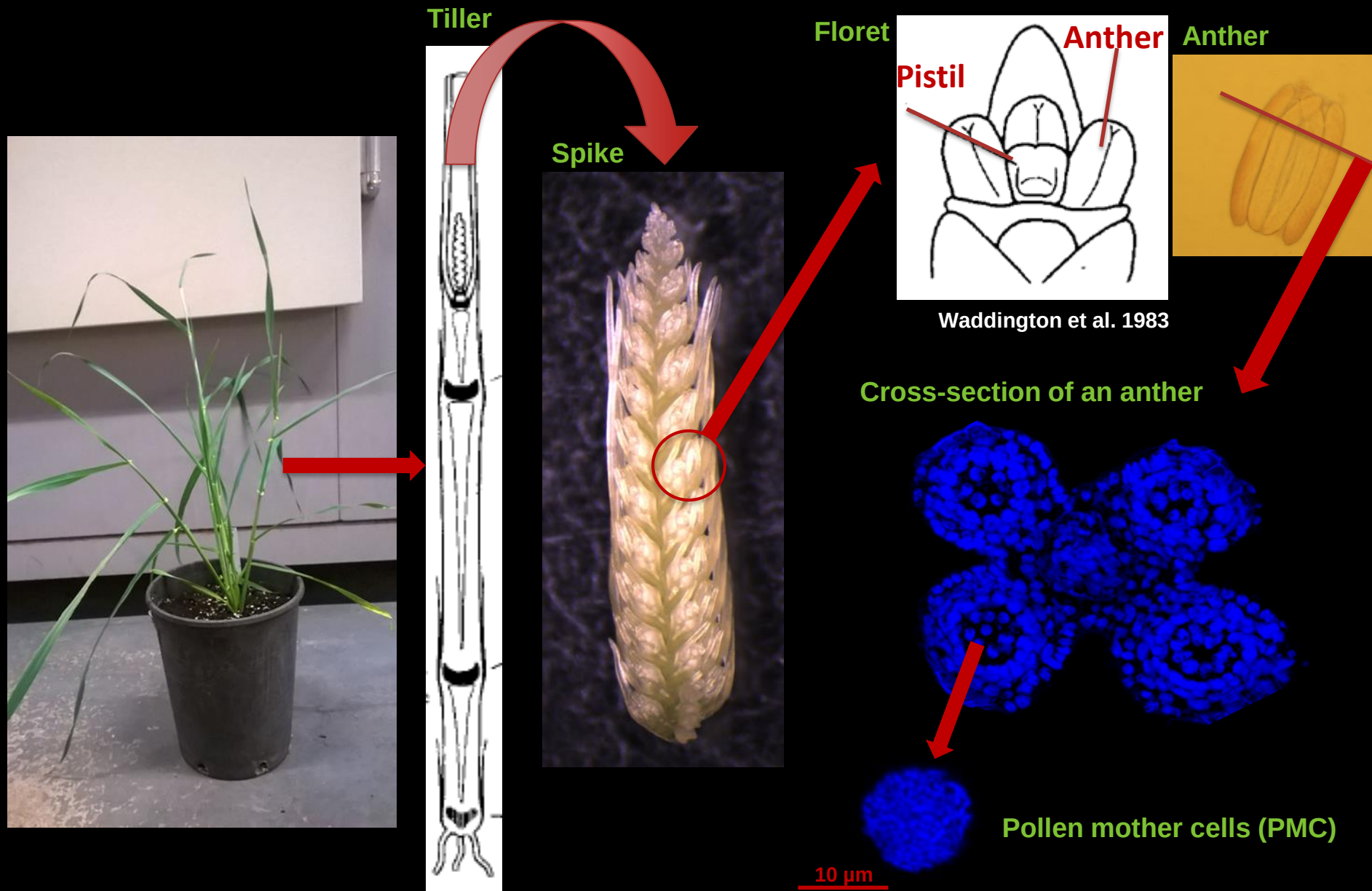
Modular trays
(Single Seed Descent)

- More than 1 tiller
- Slow growth
- Needs space in the cabinet for few plants





"Guessing" the meiotic stage of a plant



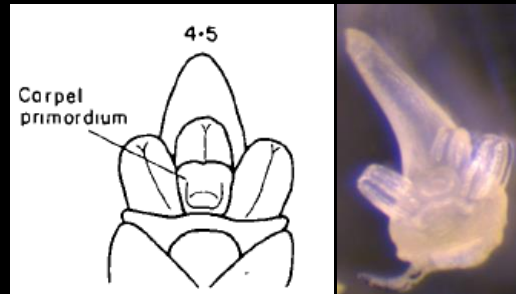
Staging work – Working with SSD trays

Staging work conducted to find the best relations to predict meiotic stages

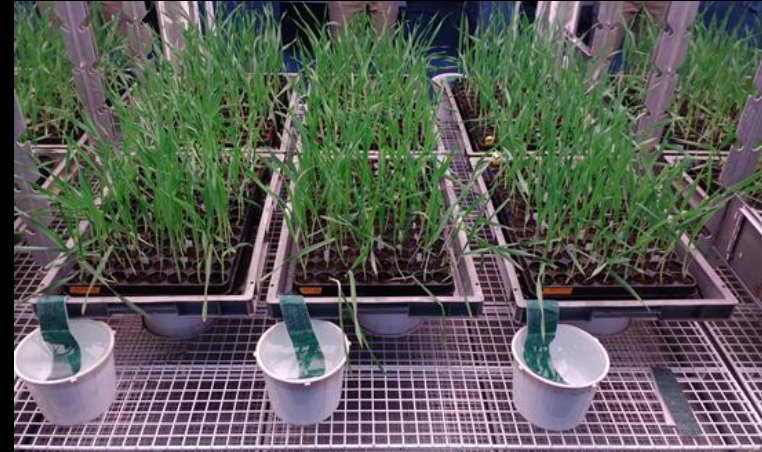
Zadok scale



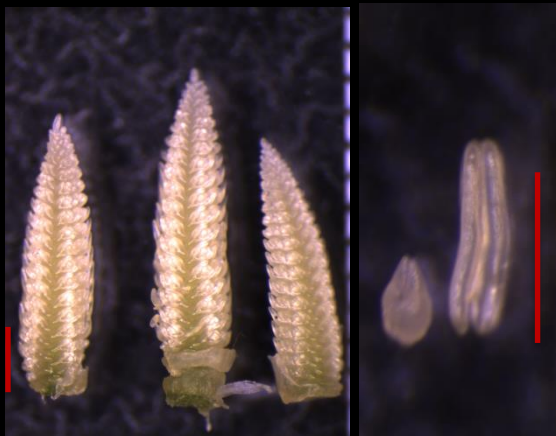
Waddington scale



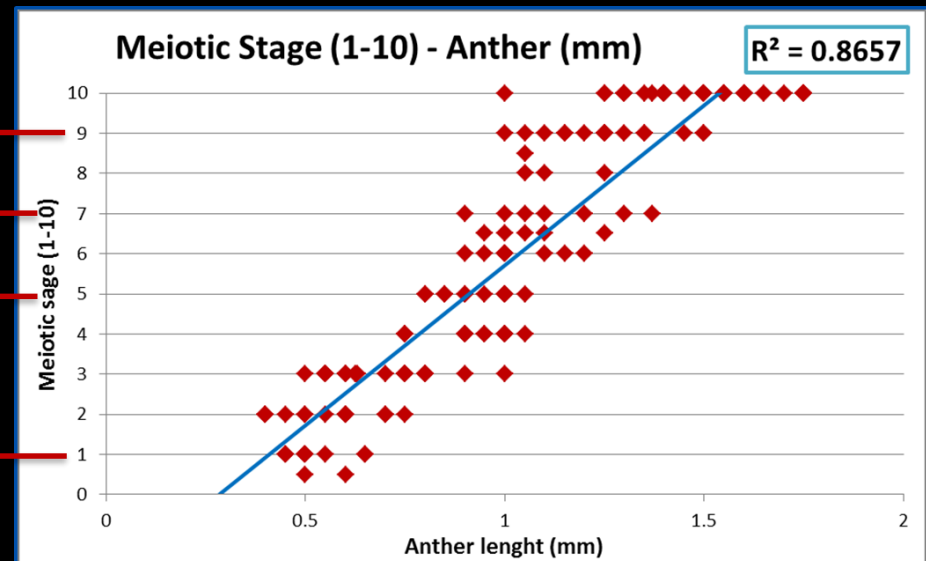
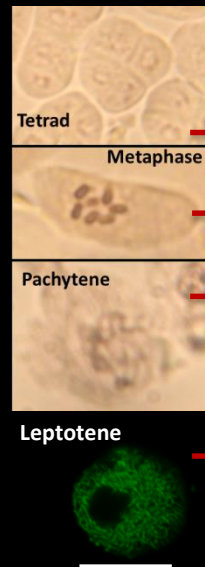
Waddington et al. 1983



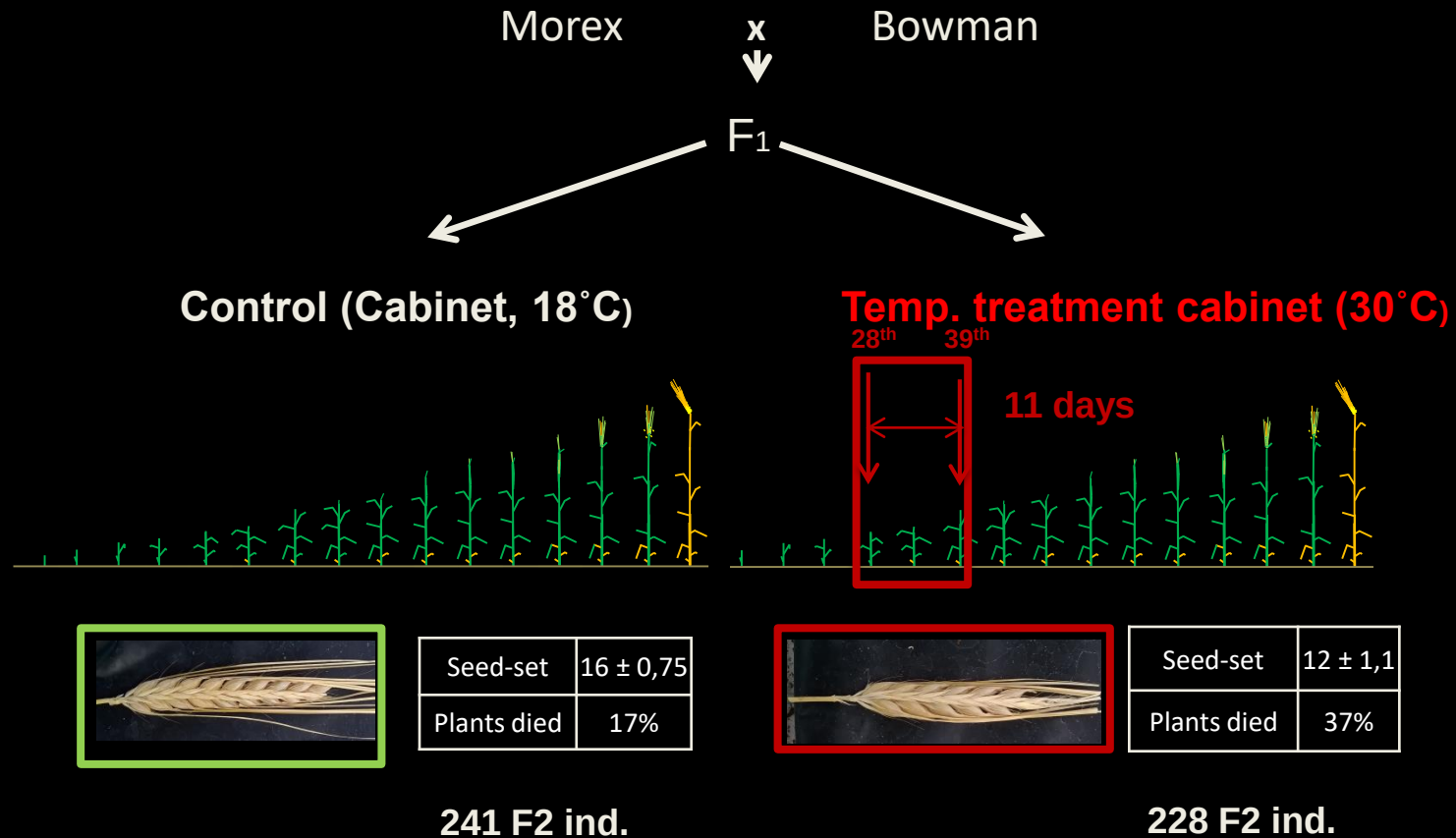
Spike size and middle anther size



Red bar: 1mm



Short Heat Treatment (30°C 11 days)

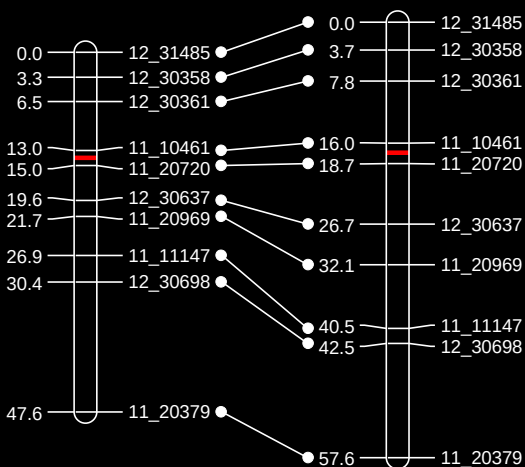


Short Heat Treatment (30°C 11 days)

Chromosome 6H Genetic map

CONTROL
n=315
18°C/16°C

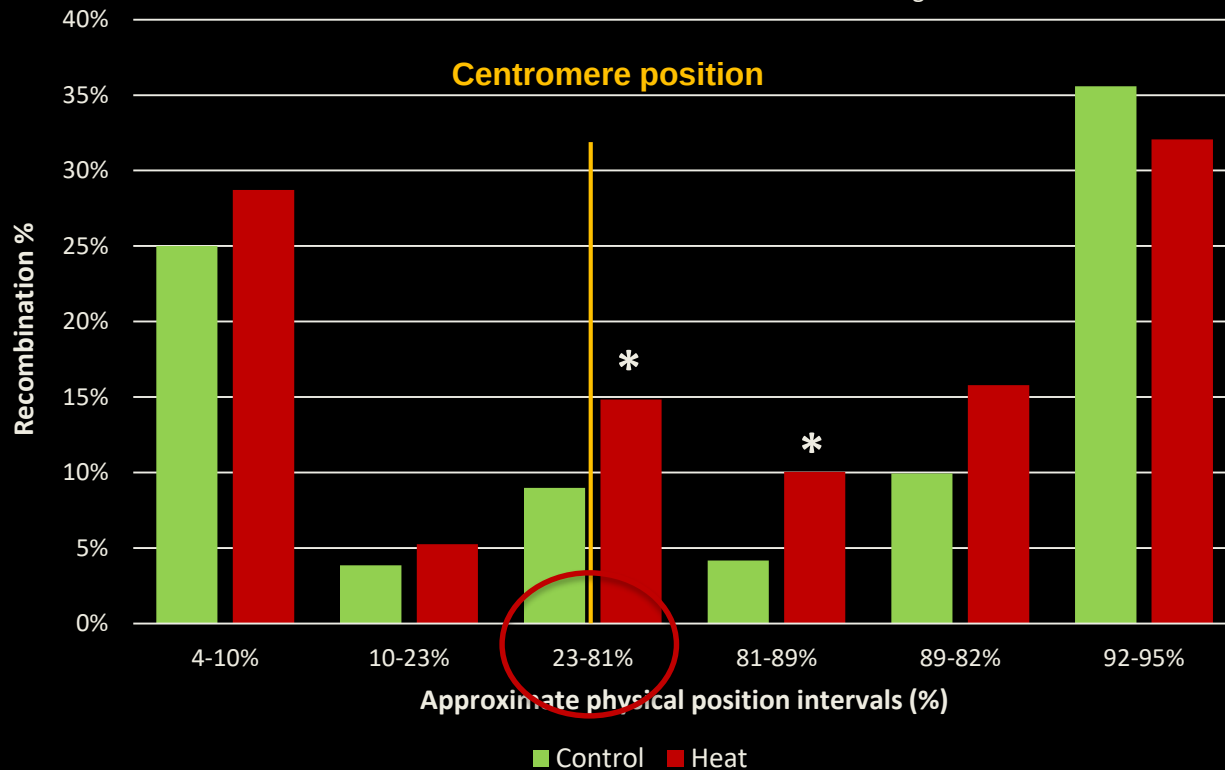
HEAT
n=209
30°C/18°C



— Approximate centromere position

Chromosome 6H – Physical map intervals

* Significant difference at 0.05



Mb intervals

24-56

56-132

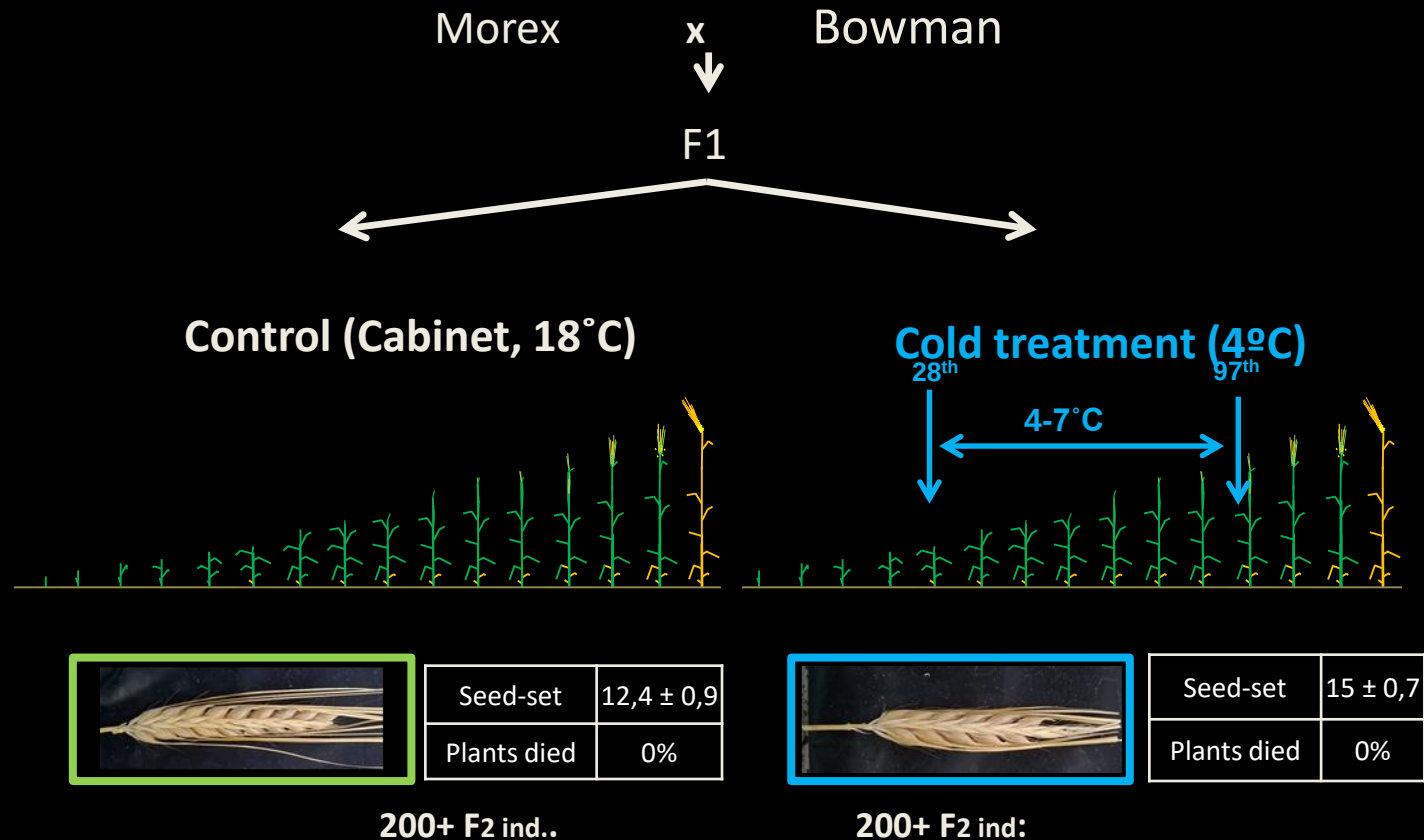
132-471

471-518

518-540

540-555

Cold Treatment (4°C 65 days)



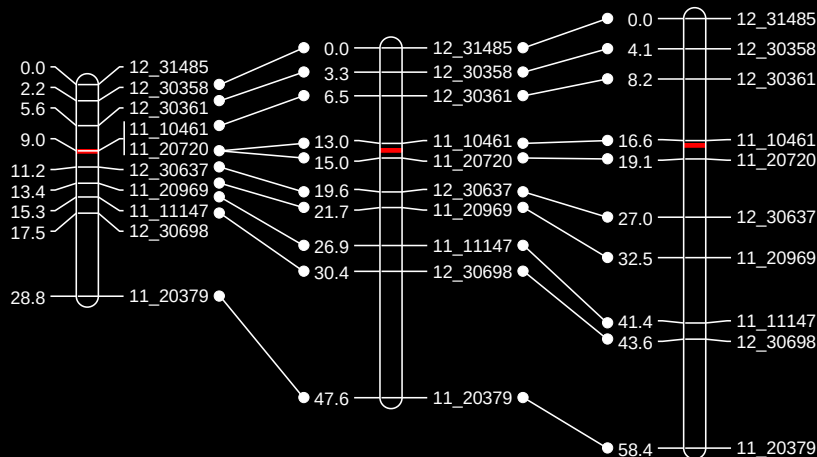
Cold Treatment (4°C 65 days)

Chromosome 6H Genetic map

COLD
n=138
4°C

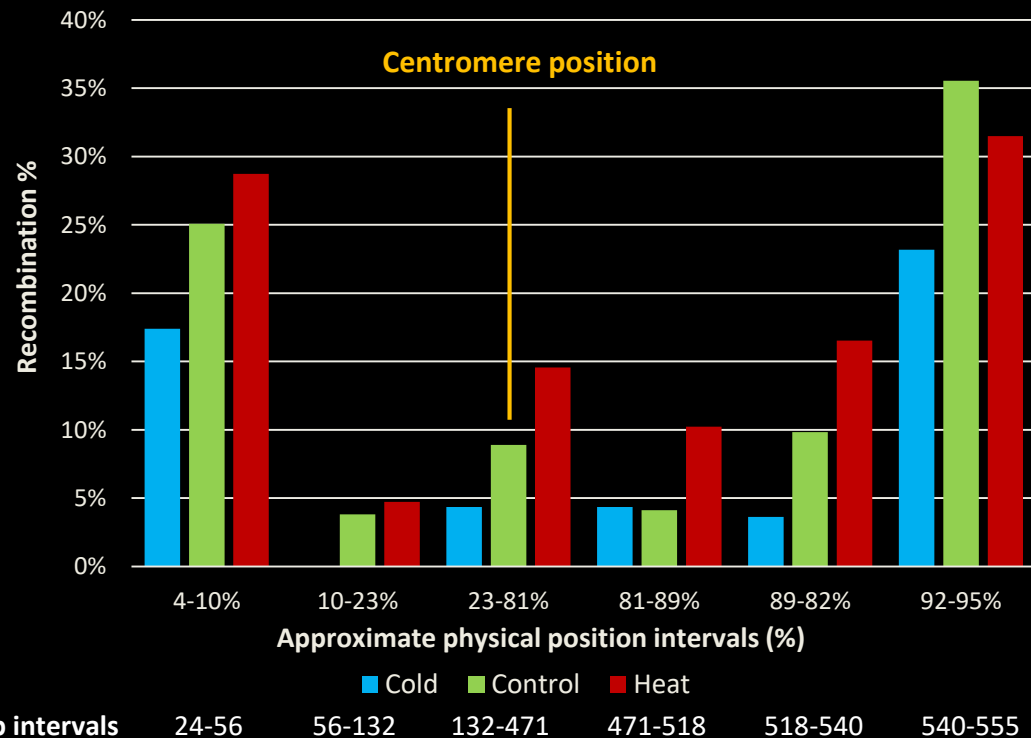
CONTROL
n=315
18°C

HEAT
n=254
30°C



— Approximate centromere position

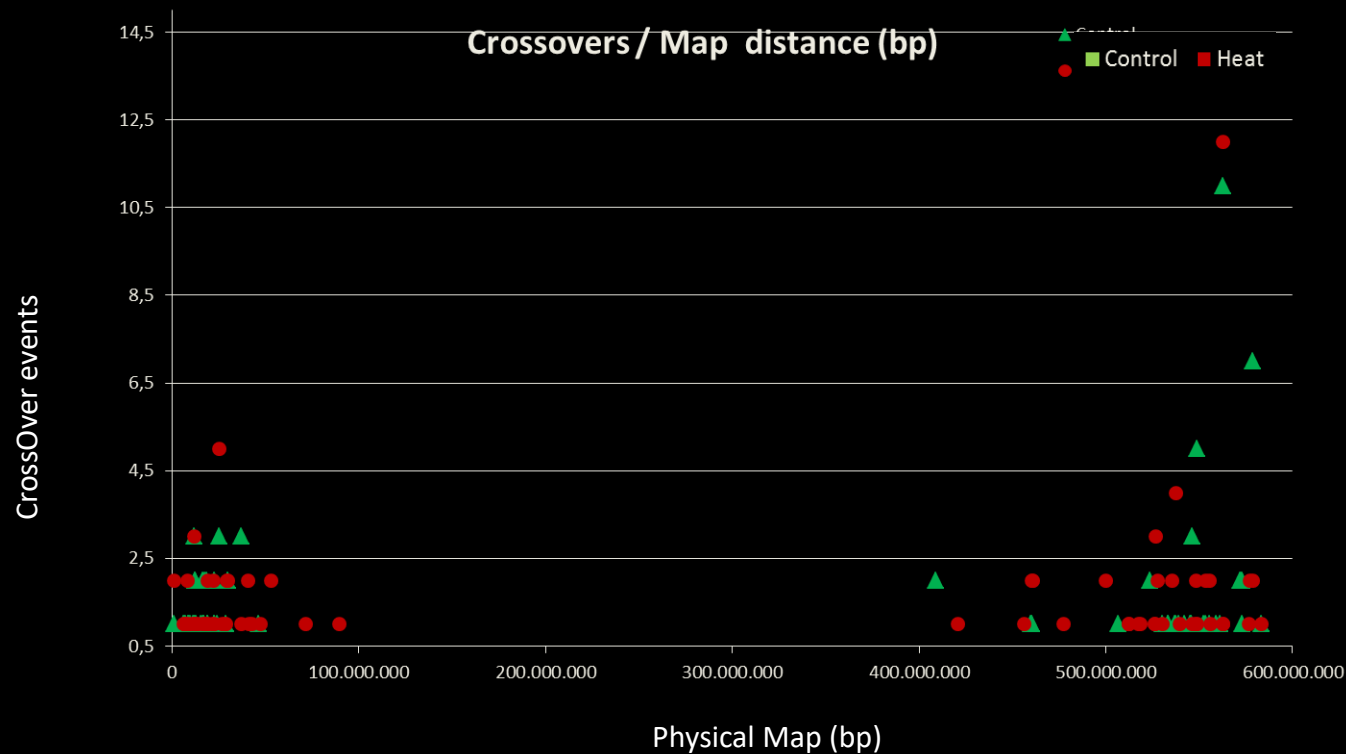
Chromosome 6H Physical map intervals





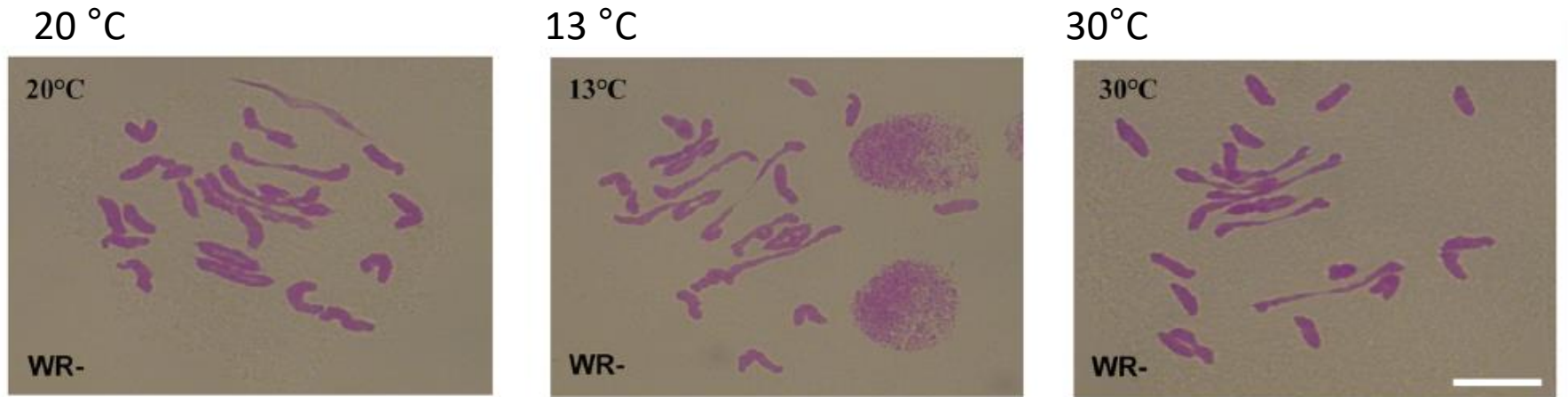
50K Genotyping - preliminary data

- 50K Illumina iSelect 46,639 SNP markers
- More accurate estimate position of CO events
- Preliminary data – chromosome 6H CO frequency and distribution



Not always the same story

Temperature stress in wheat (Graham Moore JIC)



Martin et al 2017

No. Chiasmata in Wheat - Rye Hybrids without *Ph1*

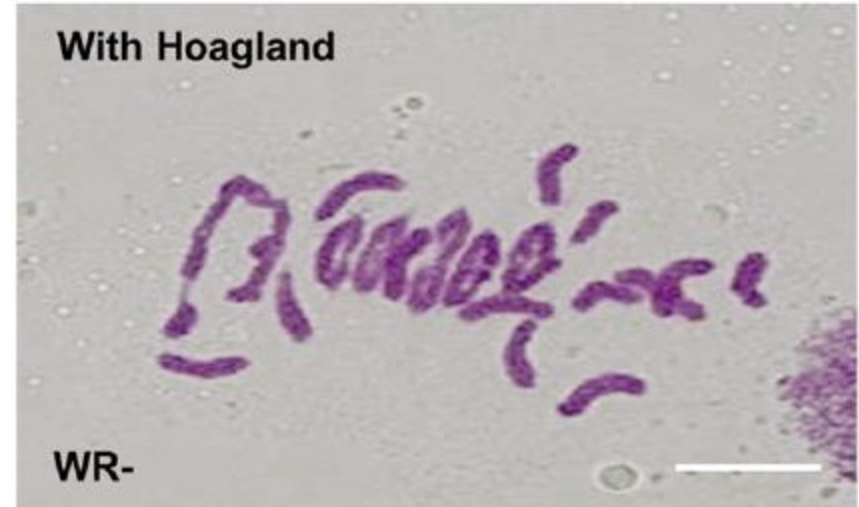
8.71 ± 0.24

11.19 ± 0.16

7.64 ± 0.22

N.B. Opposite effect observed to that in barley

Nutrient stress in wheat:



Martin et al 2017

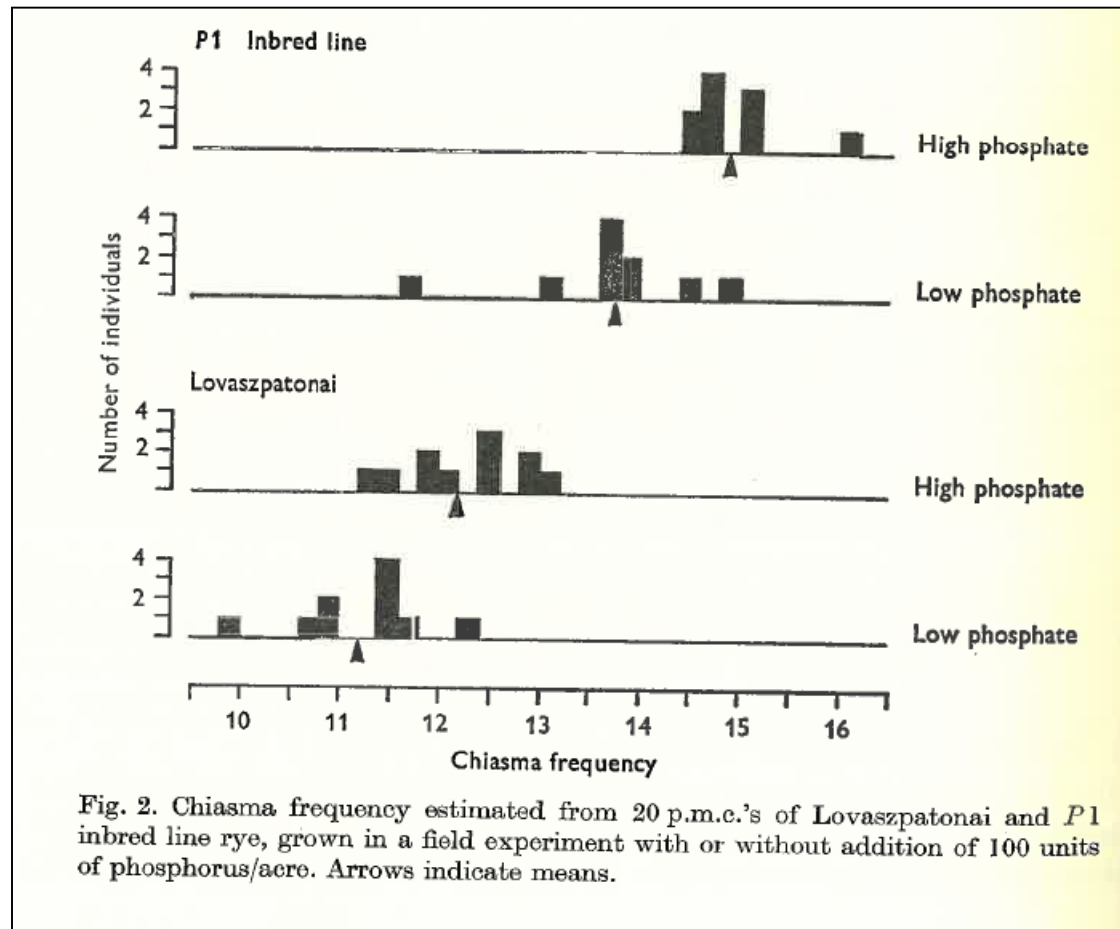
No. Chiasmata Wheat - Rye Hybrid without *Ph1*

8.71 ± 0.24

12.09 ± 0.21

Larger effect observed than that with temperature (13°C)

Phosphate stress in rye:



Bennett & Rees 1970

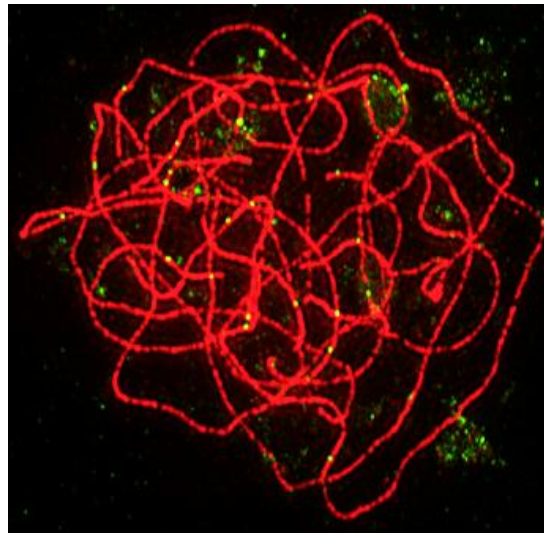
Conclusions:

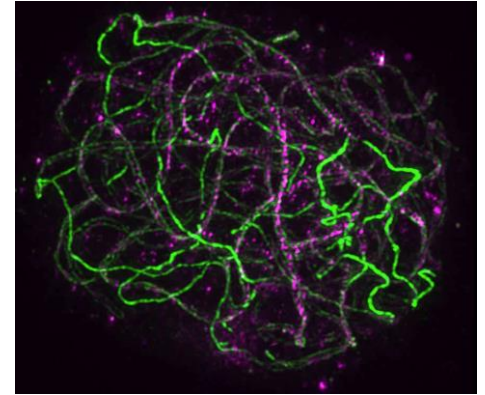
Environmental stress does effect recombination frequency and distribution in barley

Short heat stress - premeiotic/ early meiosis – potential breeding tool

Other environmental stresses particularly phosphate also have potential

Recombination phenotype is plastic and can be manipulated.





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~ **Merci** ~

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University of Aberystwyth

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University of Birmingham

Chris Franklin
Sue Armstrong



European Research Council
Established by the European Commission



Recombination pattern under genetic control

1. Evidence for genetic control in comparison of related spp.

Hordeum vulgare vs *H. bulbosum*

Triticum monococcum vs *Aegilops speltoides*

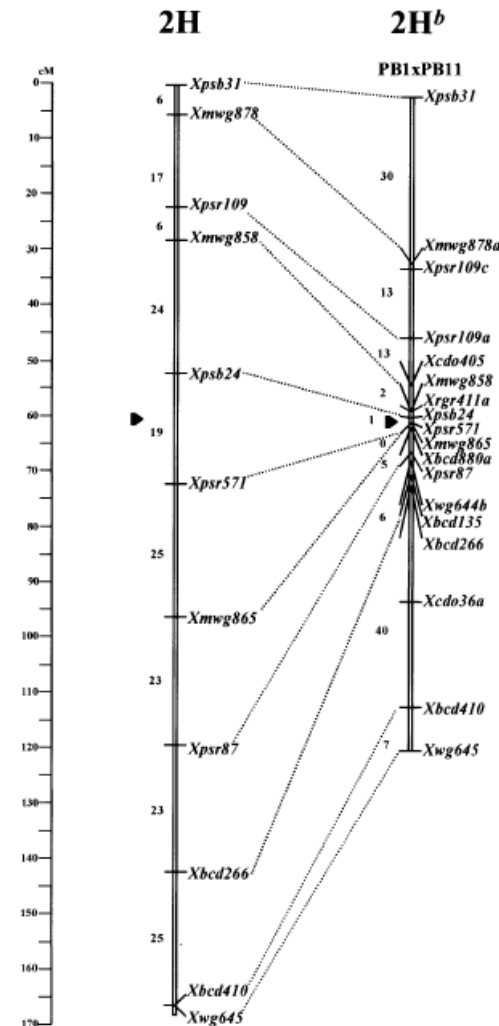
In comparison with out-breeding relative barley already has more recombination in the interstitial peri-centromeric regions

2. Limited evidence of differences in male and female recombination

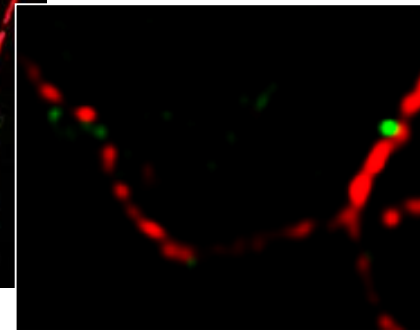
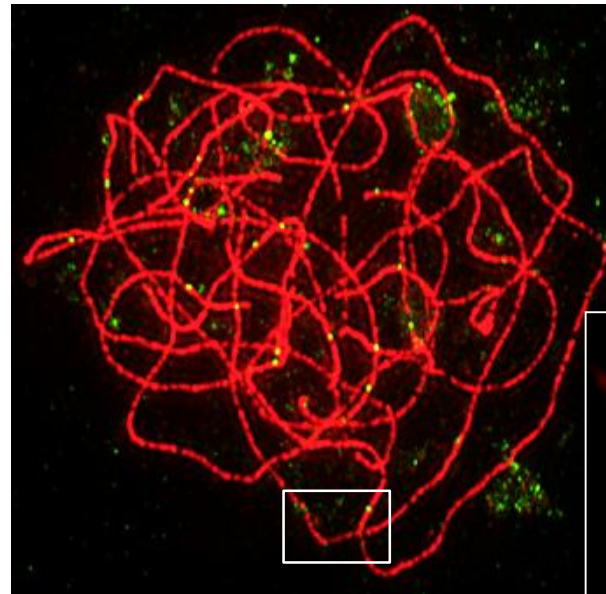
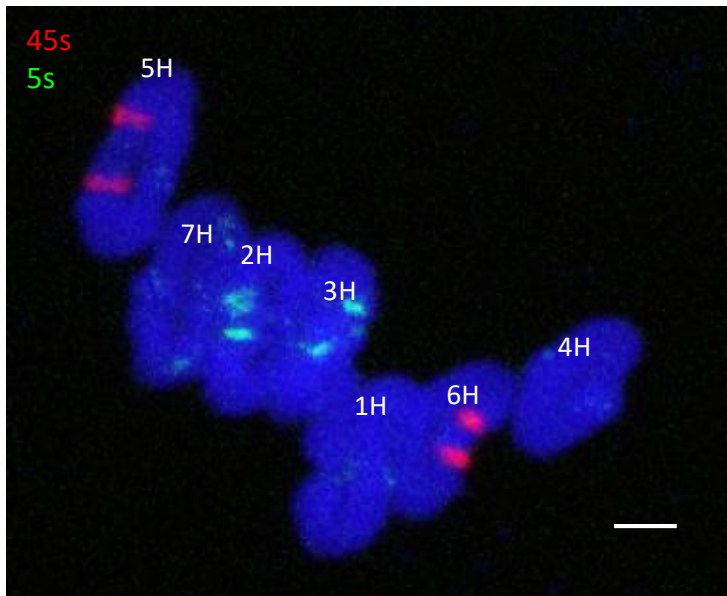
Comparison of doubled haploid populations derived from anther culture or via *H. bulbosum*

Devaux *et al.*, (1995), Cistue *et al.*, (2011)

In Triticeae v. little data – evidence in rye there is more recombination on female side (out-breeder)



Recombination- Meiotic phenotype

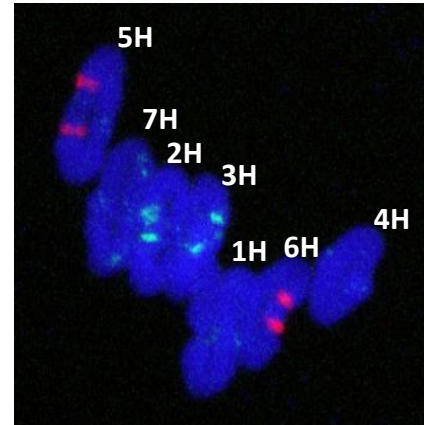
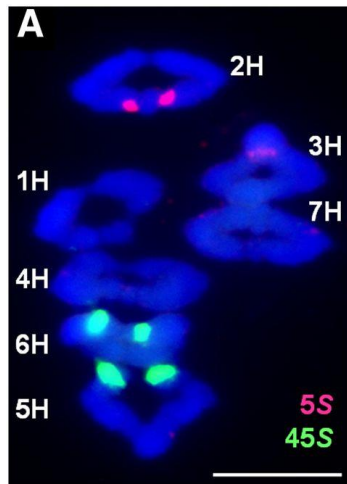


Expect 21.8 CO/cell given consensus genetic map length of barley (1090 cM)
but classically estimate ~ 14 chiasmata / cell from Metaphase I spreads

Recent work using OMX imaging - wt chiasma frequency 18.38 $\pm$ 1.35 (n=22)

HvMLH3 foci (Class I CO) MLH3 foci estimated per nucleus 20.2 ($\pm$ 3.52) (n=19)
(not trivial, requires human interpretation)

Wild type chiasmata (CO) counts



A) Individual bivalents stained with DAPI (blue) can be identified at meiotic metaphase I using the 5S (red) and 45S (green) ribosomal DNA probes with FISH. Chiasma number and position are determined by bivalent shape.

Morex 14.84 ± 0.19

Higgins et al. 2012

Golden Promise 14.18 ± 0.13 (n=60)

Barakate et al. 2014

GP ZYP1^{RNAi35} 1.4 ± 0.18 (n = 54)

Morex 13.65 ± 0.67 (n=20)

Phillips et al 2013

Bowman 13.72 ± 0.44 (n=20)

Bowman 18.4 ± 1.3

Colas et al. 2016

Wild type MLH3 (Class I CO) counts

Late HvMLH1 foci (early zygotene) + (MLH3 data not shown)

Morex	14-20 foci (n=30)	Higgins et al. 2012
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HvMLH3 foci count

Golden Promise (ev)	15 ± 2.4 (n=10)	Barakate et al. 2014
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Morex wt	19.2 ± 4.29 (n=20)	Phillips et al. 2013
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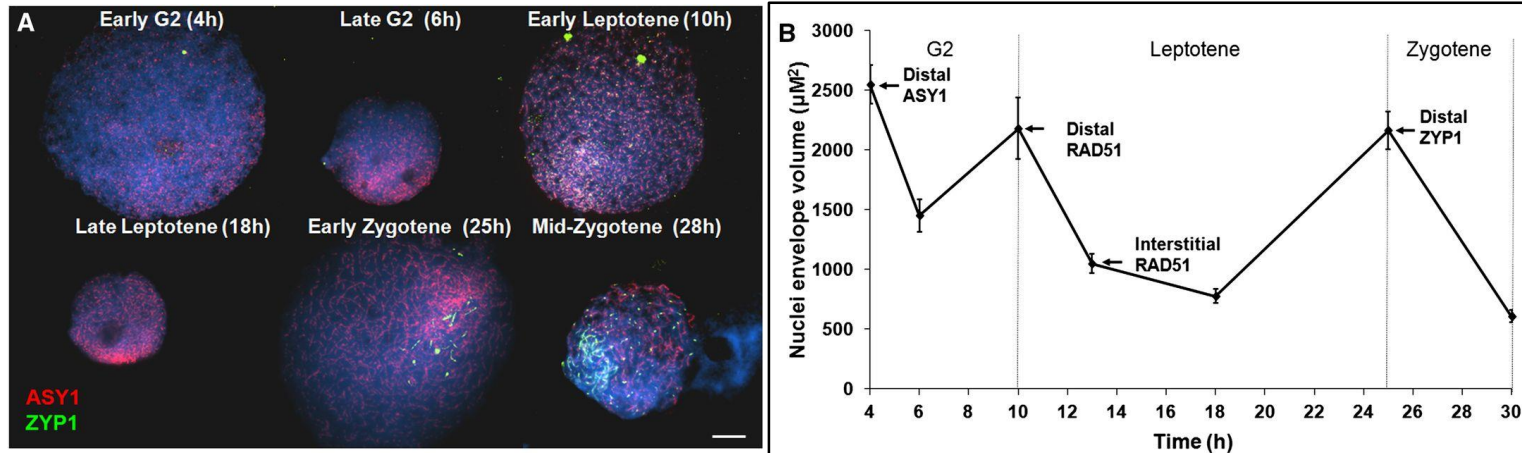
Bowman wt	14.2 ± 2.35 (n=20)	
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Steptoe x Morex F ₁ at 15°C	20.2 ± 3.52 (n=20)	Phillips et al. 2015
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at 25°C	18.6 ± 6.24 (n=20)	
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Bowman wt	20.8 ± 3.4 (n=19)	Colas et al. 2016
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Change in Distribution: Cell Cycle



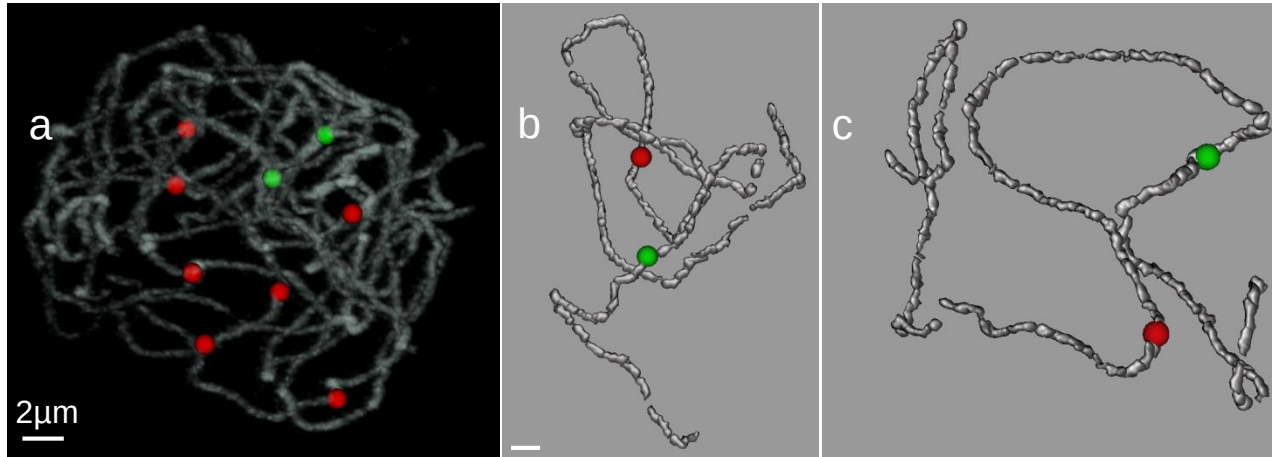
Higgins et al 2012

During prophase I, nuclei expand at transitional stages and then contract and meiotic events are in phase with this chromatin cycle

Initial distal meiotic events are synchronized with nuclei expansion periods, whereas the interstitial events occur during periods of nuclei contraction

At 30°C S phase was 4 hours shorter and the initial axis formation and loading of recombination proteins was more even

Bivalent length increase



(a) Immunolocalisation of ZYP1 in an embedded pachytene nucleus, positions of centromeres (red) and 5S (green) are shown. (b) 2H and (c) 3H bivalent isolated from nucleus shown in (a).

Synaptonemal complex (SC) of bivalents 2H and 3H in male meiosis labelled with ZYP1
Pachytene nuclei embedded in polyacrylamide

Increase in length by 30% in response to an increase in temperature 15°C - 25°C

Known correlation of SC length and crossover frequency

Increase in genetic map length due to increase in Class I CO (as in mouse) ??