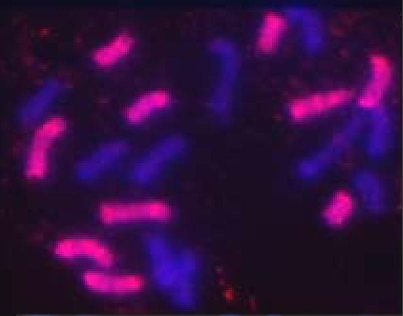
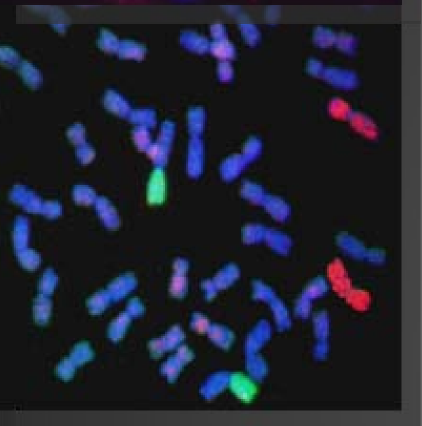

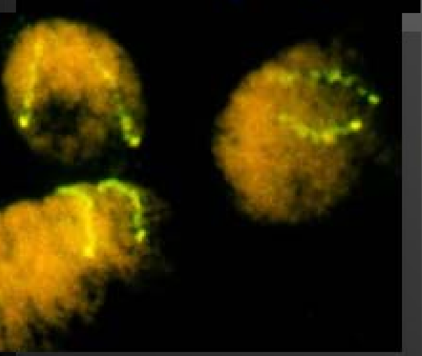

Genomic *in situ* hybridization to understand hybrid genomes

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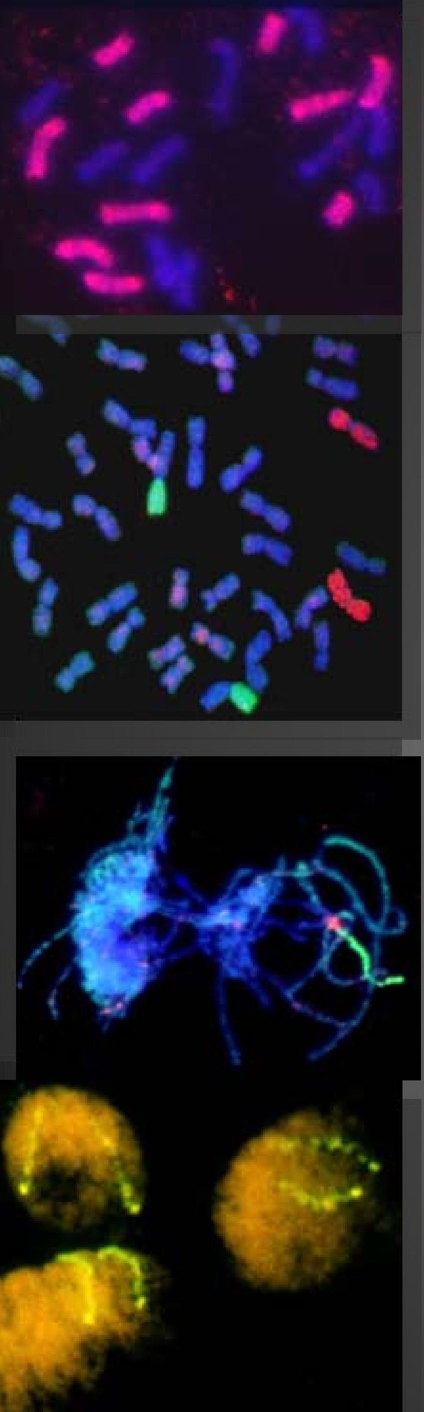
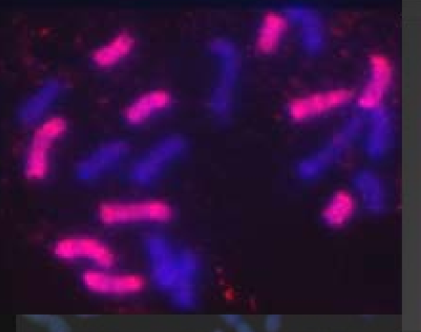
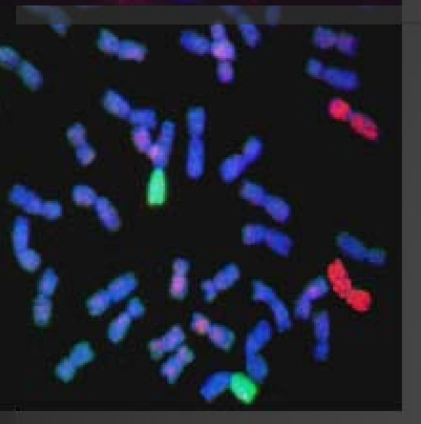


GENOMIC *IN SITU* HYBRIDIZATION GISH

- Background and history
  - What is it?
  - What can it do?
- Applications
  - Examples from our lab
    - Wheat
    - Brassica
    - Crocus



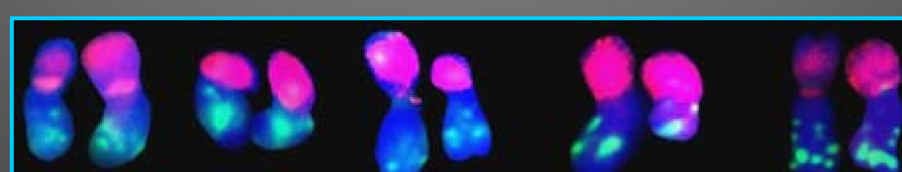


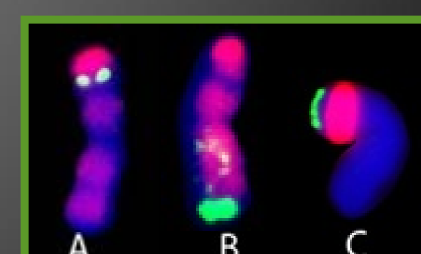
GENOMIC *IN SITU* HYBRIDIZATION GISH

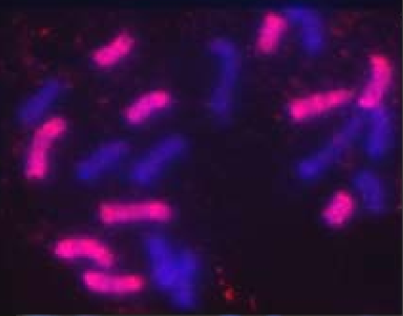
- Uses total genomic DNA as a probe for *in situ hybridization* to chromosomes and nuclei
- Identifies origin of chromatin
- Parental origin of hybrids
  - Autopolyploidy

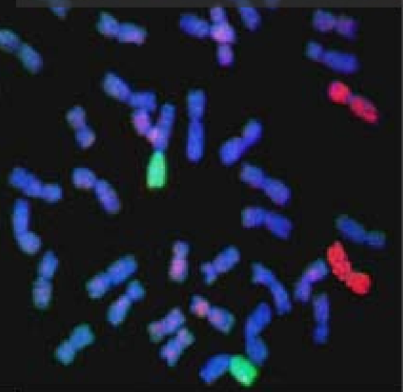
Can be combined with other probes, most often repetitive DNA probes

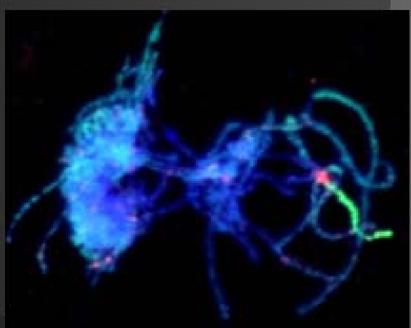
- Alien chromatin in breeding lines
  - Size and origin

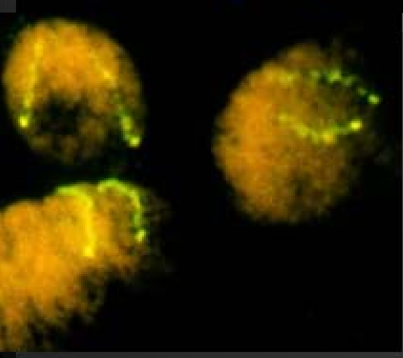






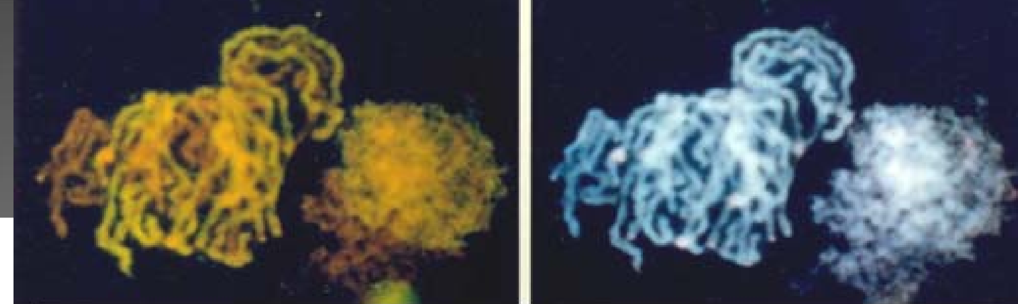






GENOMIC *IN SITU* HYBRIDIZATION GISH

- Parental origin of hybrids
  - Auto or allo-poliploidy
- Alien chromatin in breeding lines
  - Size and origin
  - Recipient chromosome
- Meiosis and chromosome pairing
- Interphase cytogenetics



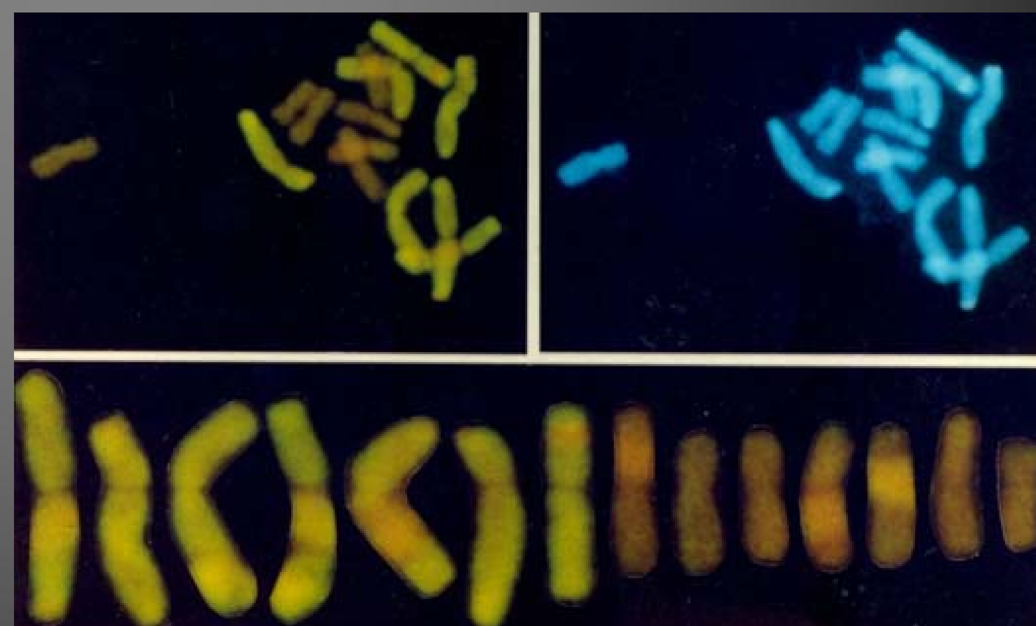
Annals of Botany **64**, 315–324, 1989

In Situ Localization of Parental Genomes in a Wide Hybrid

T. SCHWARZACHER*, A. R. LEITCH, M. D. BENNETT† and J. S. HESLOP-HARRISON

Cambridge Laboratory, Institute of Plant Science Research, Trumpington, Cambridge CB2 2JB, UK

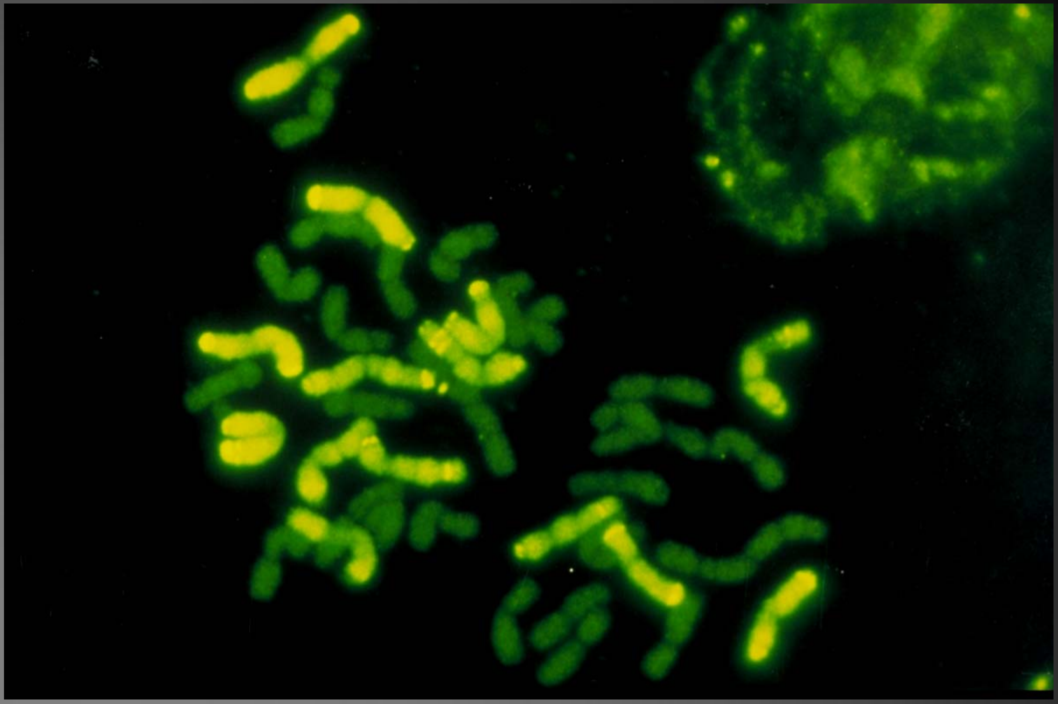
Accepted: 7 March 1989

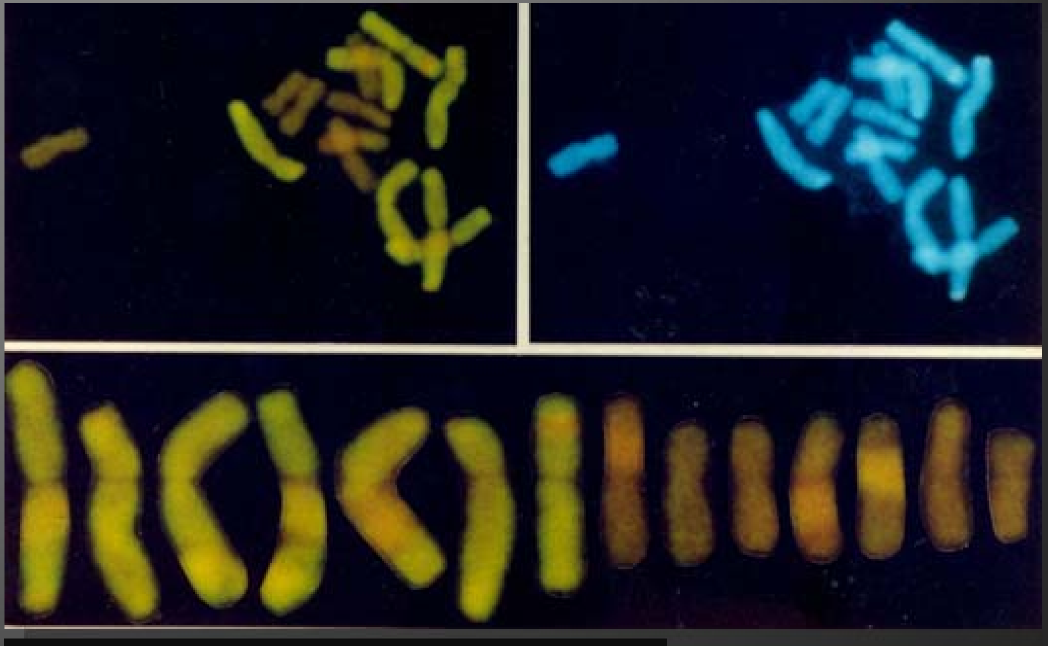


Addition of unlabelled, cold total genomic DNA to block common sequences between the parental genomes

Heslop-Harrison, J.S., Schwarzacher, T., Leitch, A.R., Anamthawat-Jonsson, K. and Bennett, M.D. (1988) A method of identifying DNA sequences in chromosomes of plants. European Patent Application 8828130.8. December 8, 1988. Early Publication, June 8, 1990.

Anamthawat-Jonsson, K., Schwarzacher, T., Leitch, A.R., Bennett, M.D. and Heslop-Harrison, J.S. (1990) Discrimination between closely related Triticeae species using genomic DNA as a probe. Theoretical and Applied Genetics 79, 721-728.





Theor Appl Genet (1992) 84: 778–786

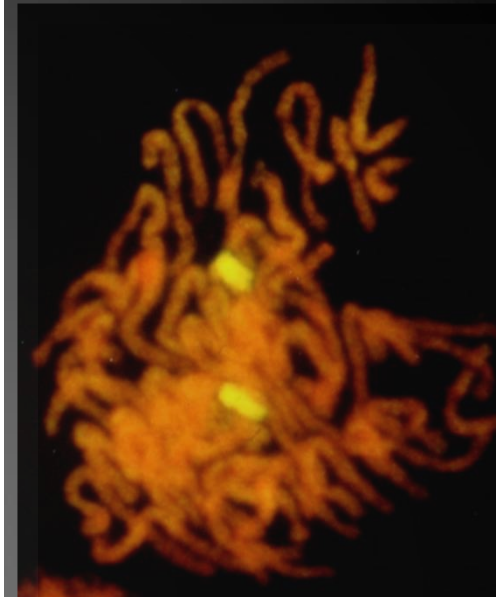
THEORETICAL AND APPLIED GENETICS

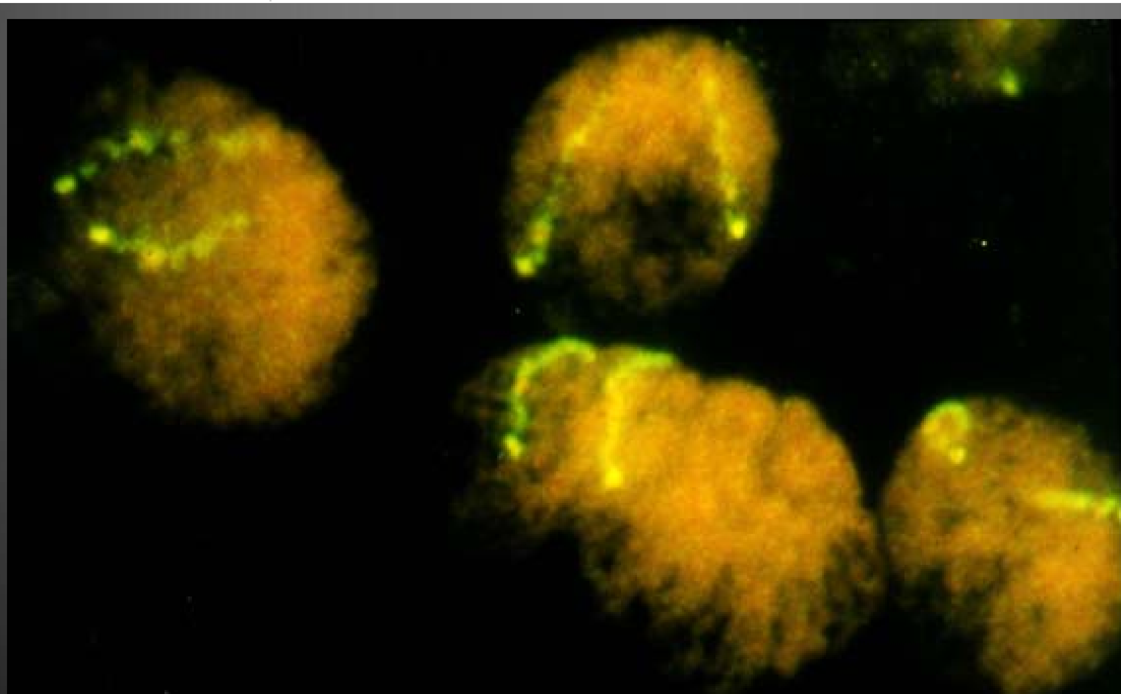
Genomic in situ hybridization to identify alien chromosomes and chromosome segments in wheat

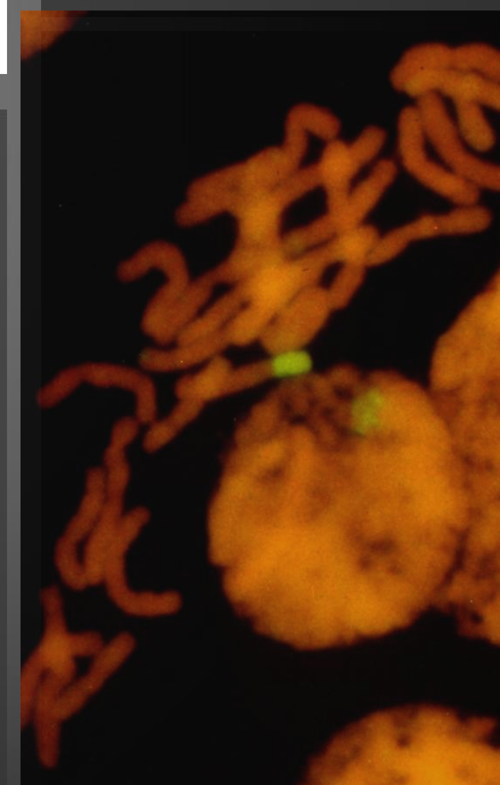
T. Schwarzacher[1], K. Anamthawat-Jónsson[1,2], G. E. Harrison[1], A. K. M. R. Islam[3], J. Z. Jia[4], I. P. King[1], A. R. Leitch[1,5], T. E. Miller[1], S. M. Reader[1], W. J. Rogers[1,6], M. Shi[1] and J. S. Heslop-Harrison[1]

[1] John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UJ, UK
[2] Agricultural Research Institute, Keldnaholt, Reykjavik, 112, Iceland
[3] Waite Institute, University of Adelaide, Glen Osmond SA 5064, Australia
[4] The Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[5] Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK
[6] School of Life Sciences, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK

Received December 12, 1991; Accepted February 26, 1992







EXAMPLES WHERE GISH HAS BEEN SUCCESSFUL

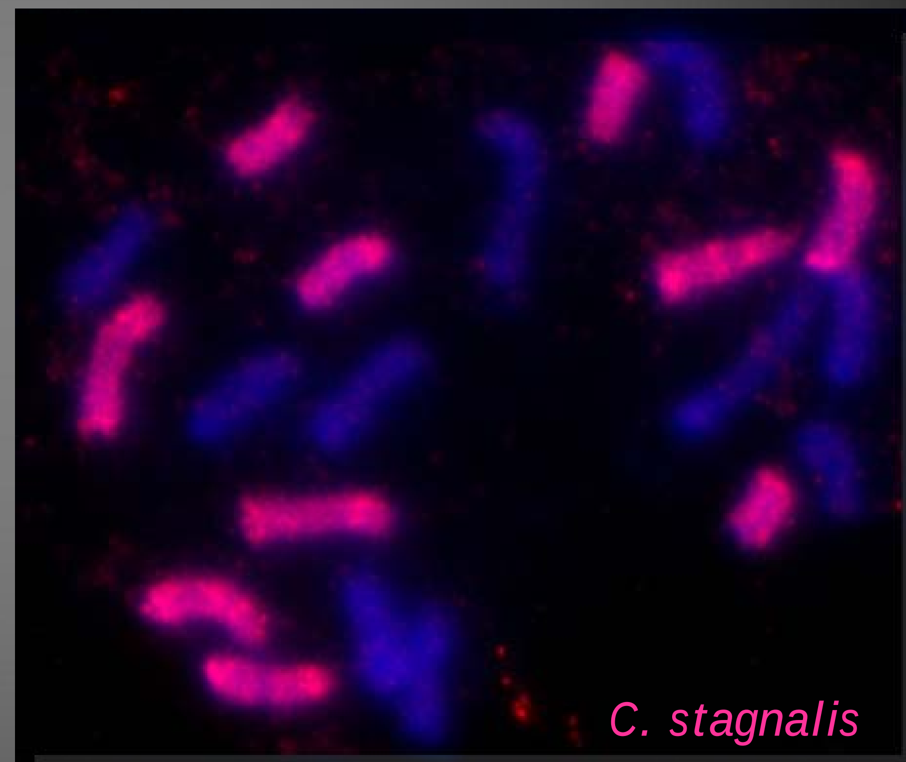
- Triticae
  - *Triticum, Aegilops, Hordeum, Haynaldia, Thinopyrum, Secale, Hystrix, Leymus, Agropyron, Elymus, Elytrigia*
- *Brachypodium*
- *Oryza* genomes
- *Zea mays*
- *Pennesitum*
- *Tripsacum*
- *Saccharum*
- *Avena*
- *Lolium* and *Festuca* hybrids *Festulpia*
- *Eleusine*
- *Alstroemeria*
- *Aloe*
- *Lilium*
- *Allium*
- *Crocus*
- Tulipa
- *Musa* genomes
- Asteraceae
  - *Dahlia*
  - *Chrysanthemum, Dendranthema and Argyranthemum*
- Brassicaceae
  - Brassica species, alien and hybrids with *Eruca, Orchyophragmus, Sinapis, Raphanus Brassica, Lesquerella fendleri*, Arabidopsis species/hybrids
- Solanaceae
  - *Solanum*, potato, tomato
- Fabaceae
  - *Arachis*
  - *Medicago*
- *Coffea arabica*
- Gossypium
- *Rubus*
- *Beta*
- *Zingeria*
- *Setaria*
- *Phalaenopsis*

Calitriche platycarpa (2n=20)
Water starworts

Savidge (1960): *allo-tetraploid origin from C. stagnalis and C. cophocarpa* (both 2n=10)

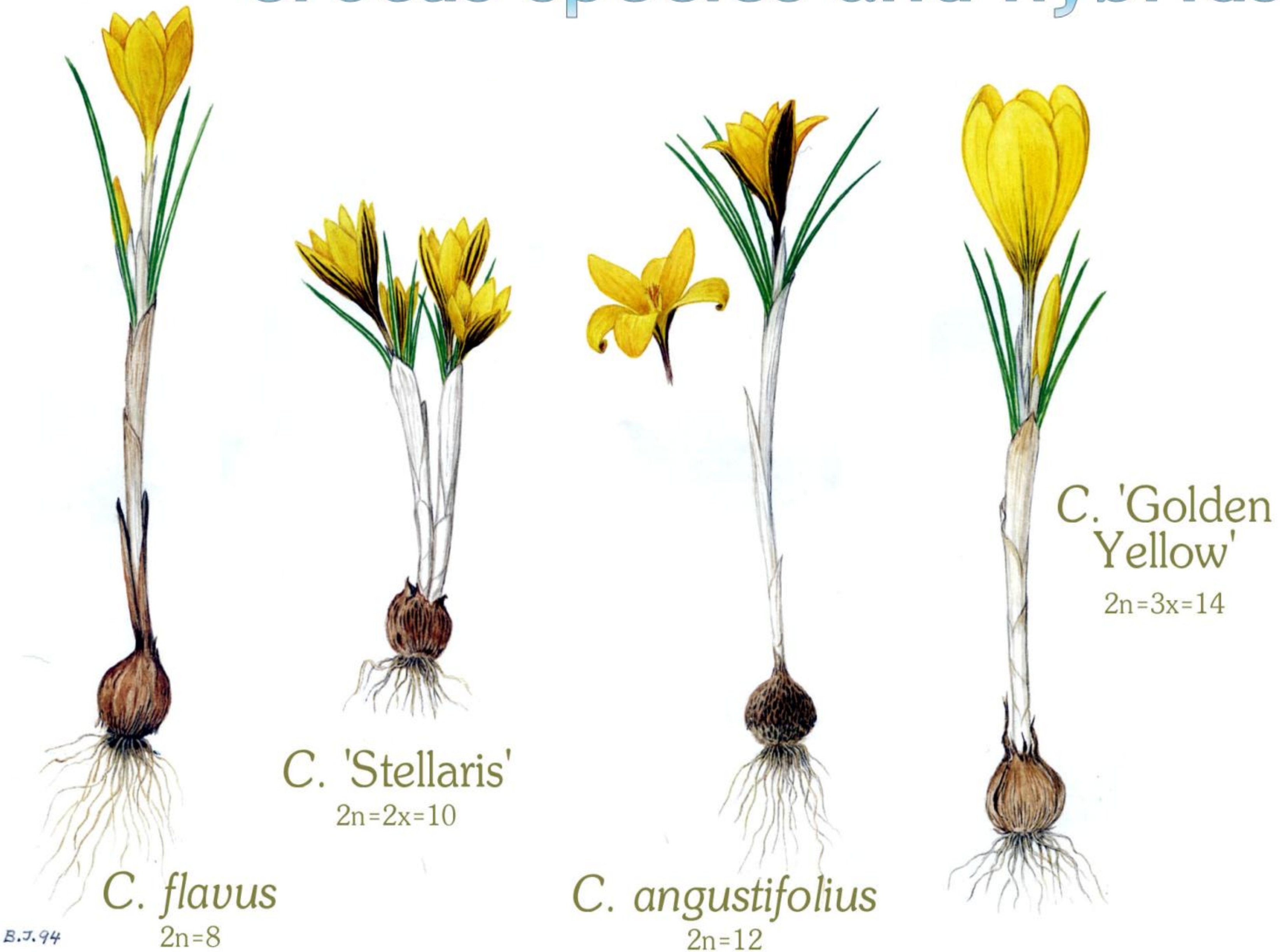
Schotsman (1967):
auto-tetraploid from *C. cophocarpa*

British Species	2n
Callitriche hermaphroditica L.	6
Callitriche truncata Guss.	6
Callitriche stagnalis Scop.	10
Callitriche obtusangula Le Gall	10
Callitriche platycarpa Kütz.	20
Callitriche palustris L.	20
Callitriche brutia Petagna	28
Callitriche hamulata Kütz.	38



Gornall, Johnson and Schwarzacher 2004

Crocus species and hybrids



‘GOLDEN YELLOW’ CROCUS

2n=3x=14

6 chr from *Crocus angustifolius*

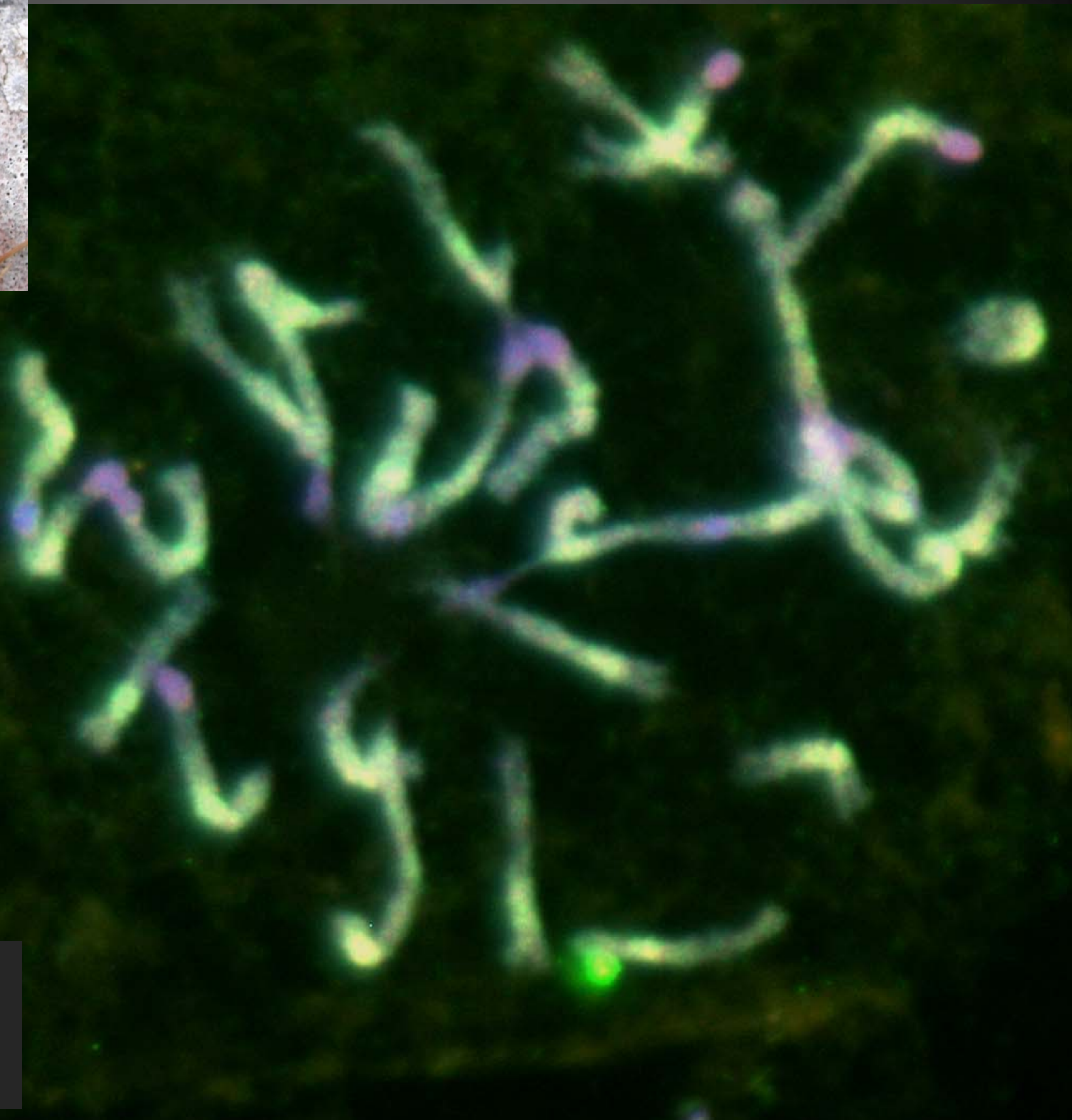
2x4 chr from *Crocus flavus*


www.CrocusBank.org

Saffron

Crocus sativa

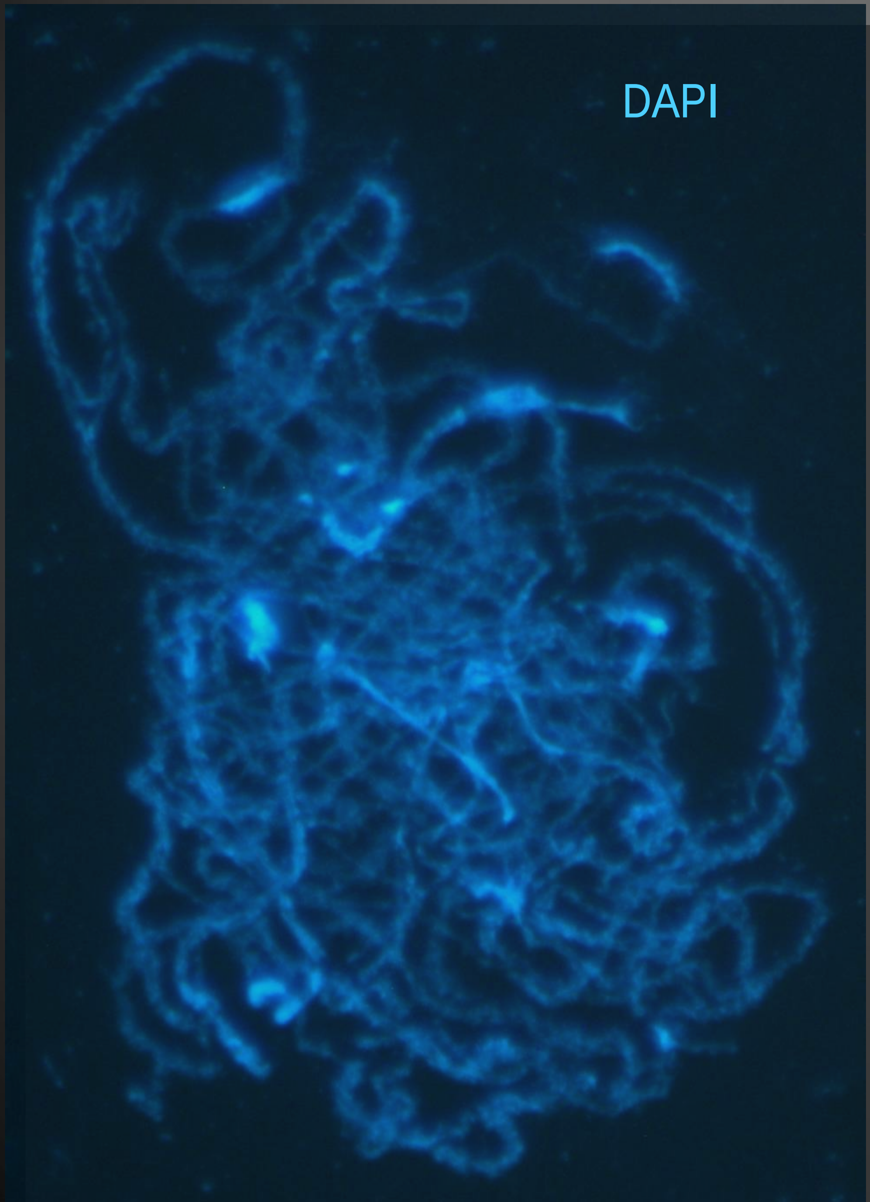
$2n=3x=24$

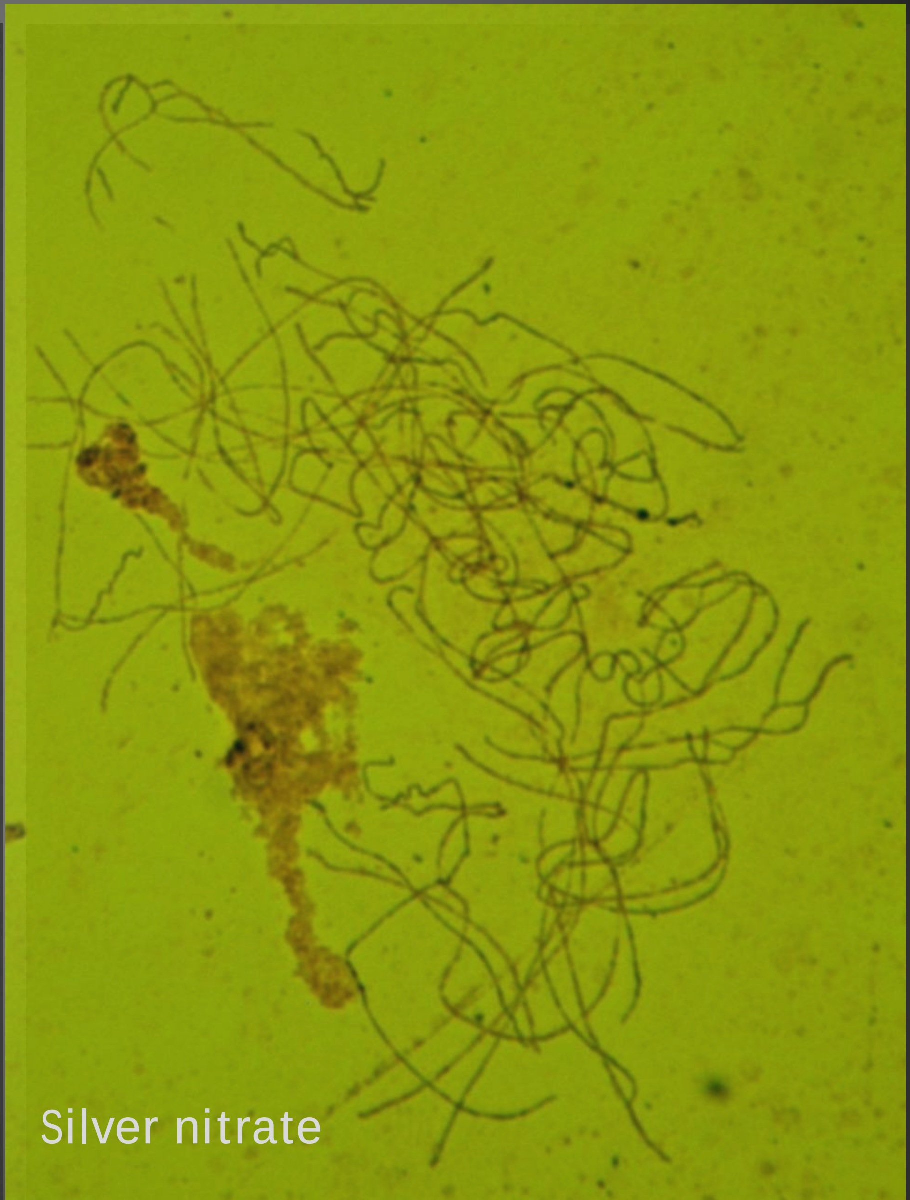


C. cartwrightianus green

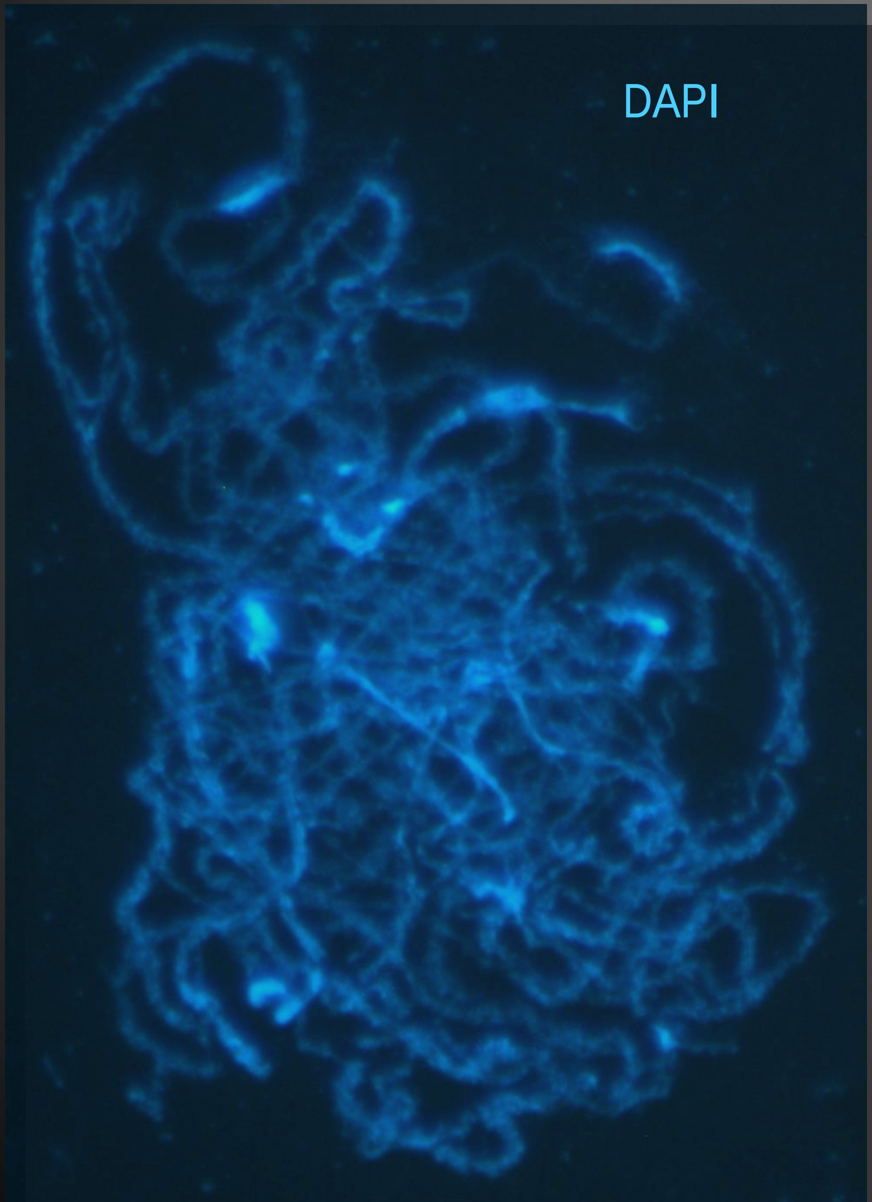
C. *thomasii* red

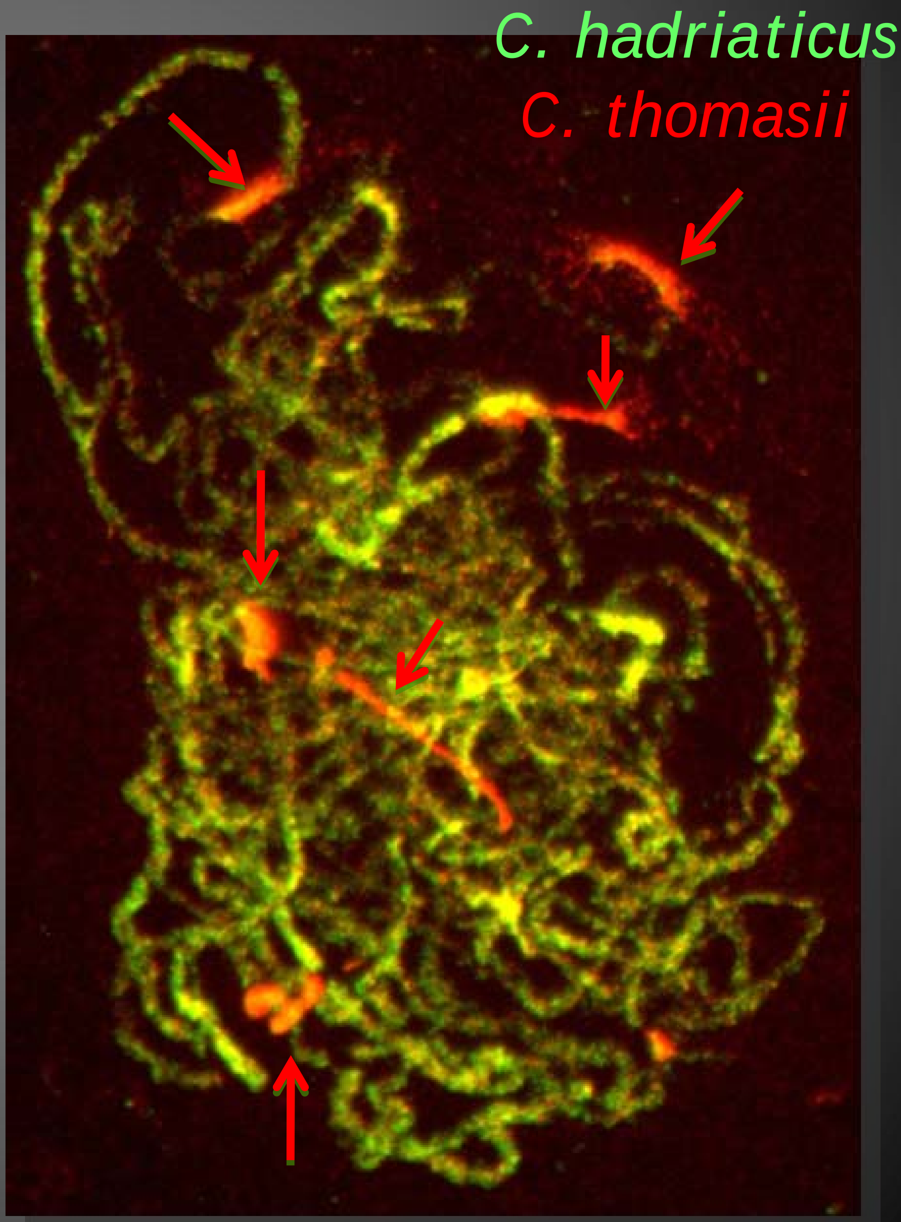
Crocus sativus Pachytene



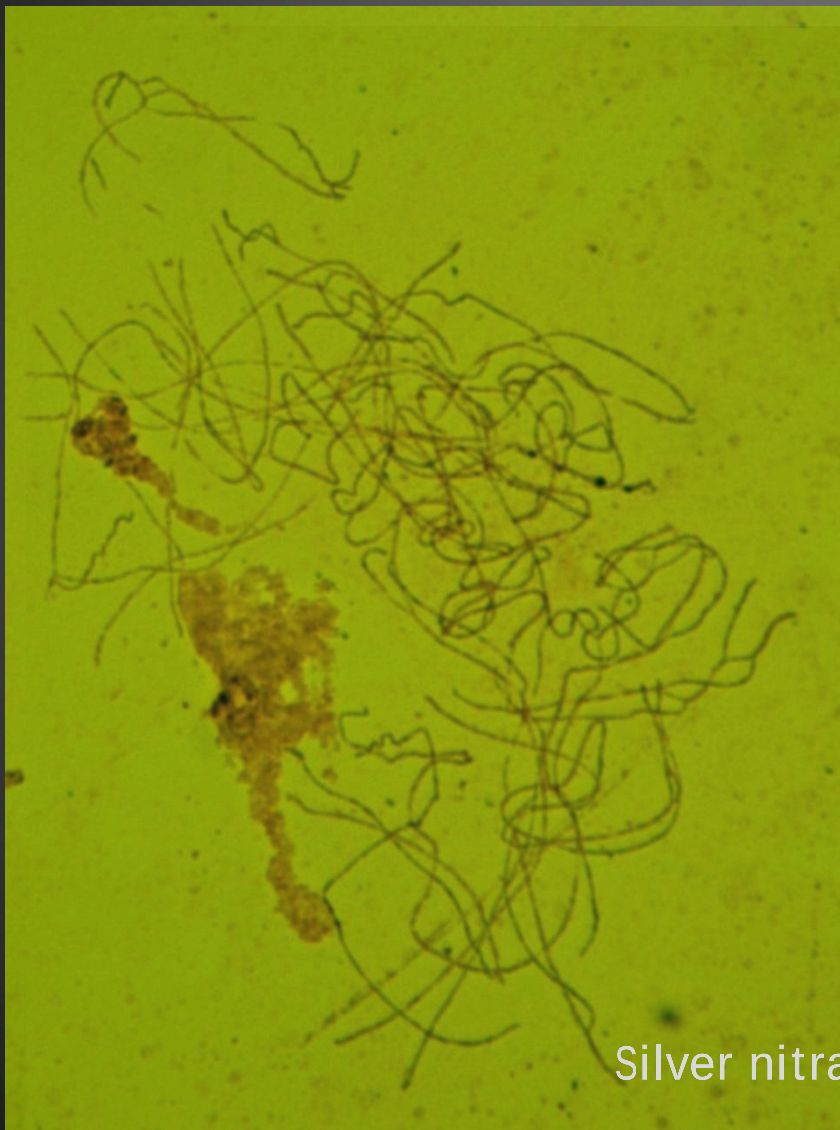


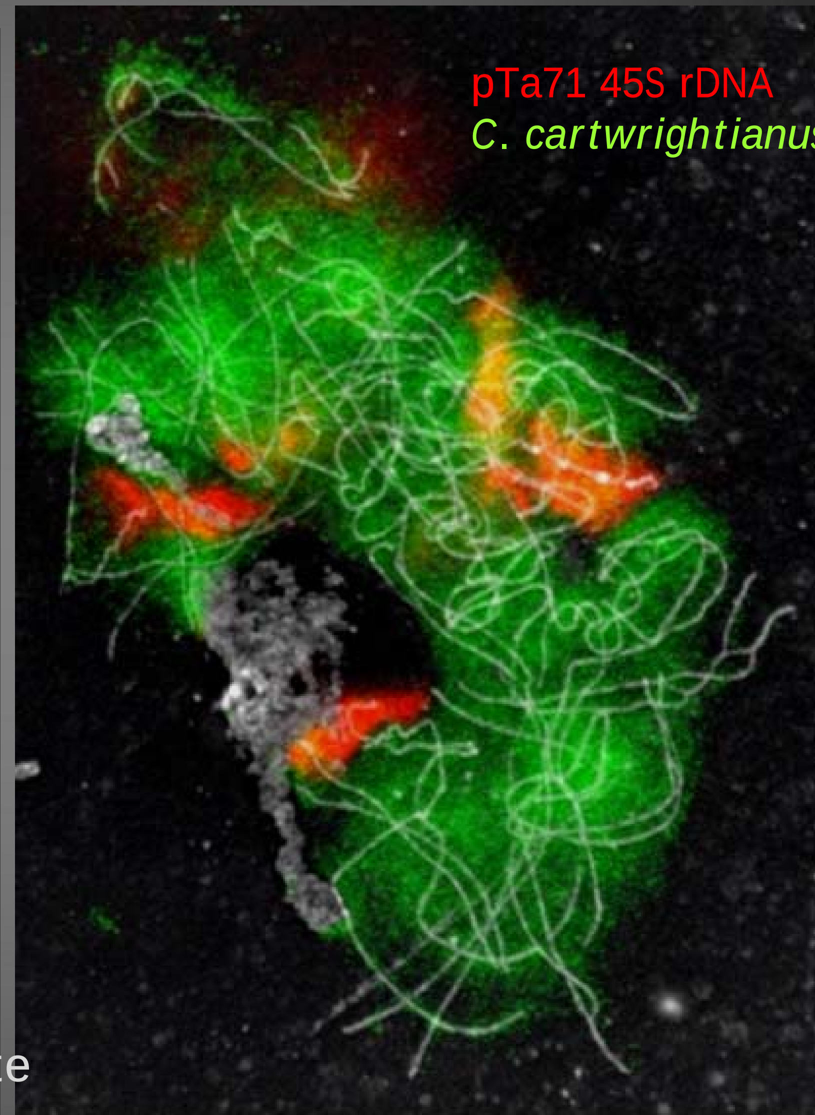
Crocus sativus Pachytene





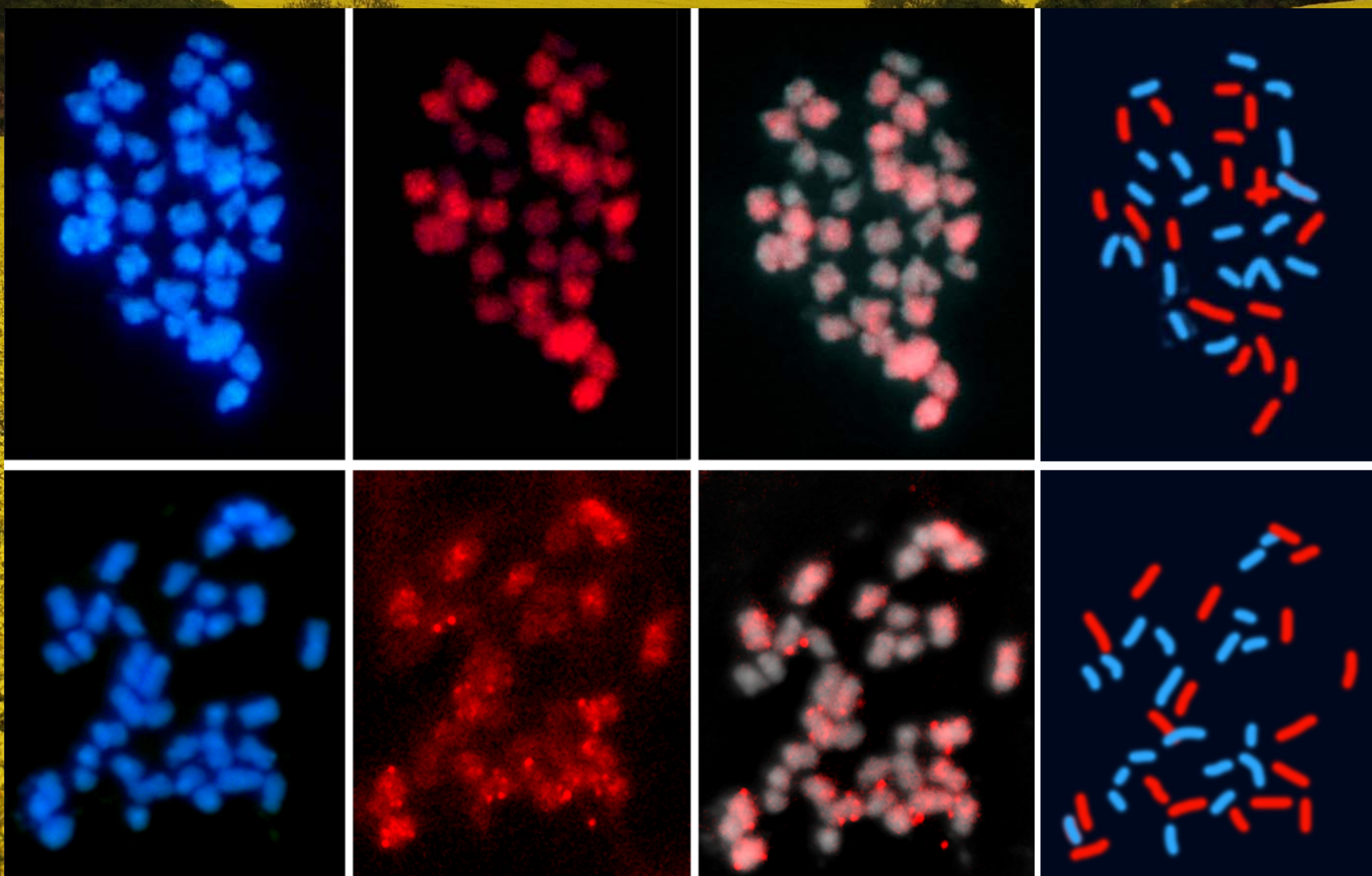
Crocus sativus Pachytene





Rapeseed *B. napus* (AACC, 2n=4x=38) - hybridized with C-genome CACTA element red

B. oleracea (CC, 2n=2x=18)   *B. rapa* (AA, 2n=2x=20)

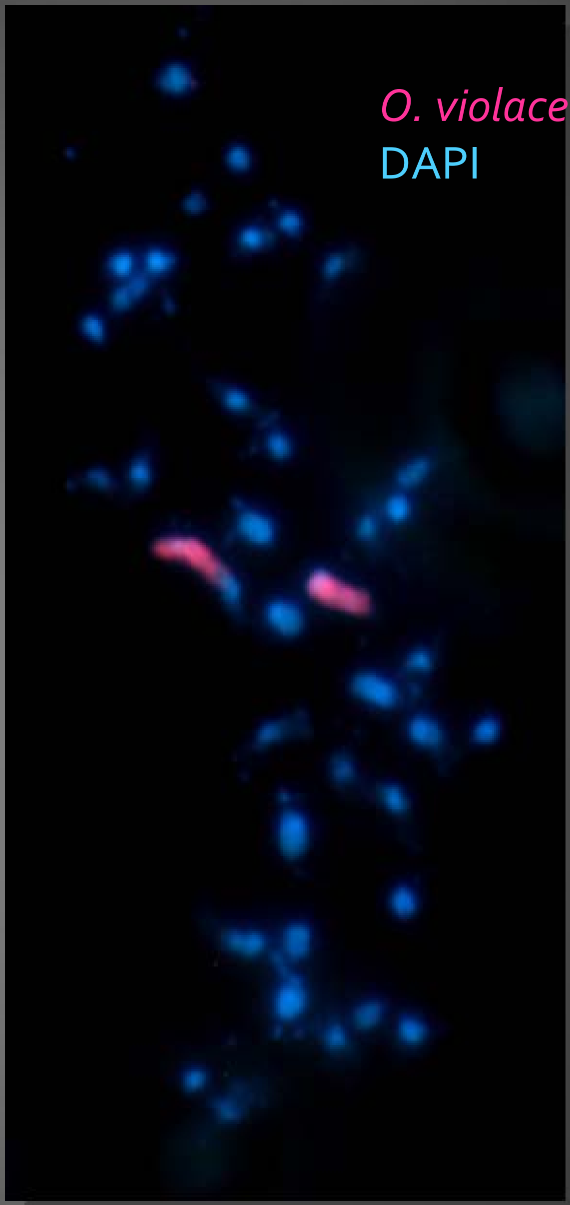


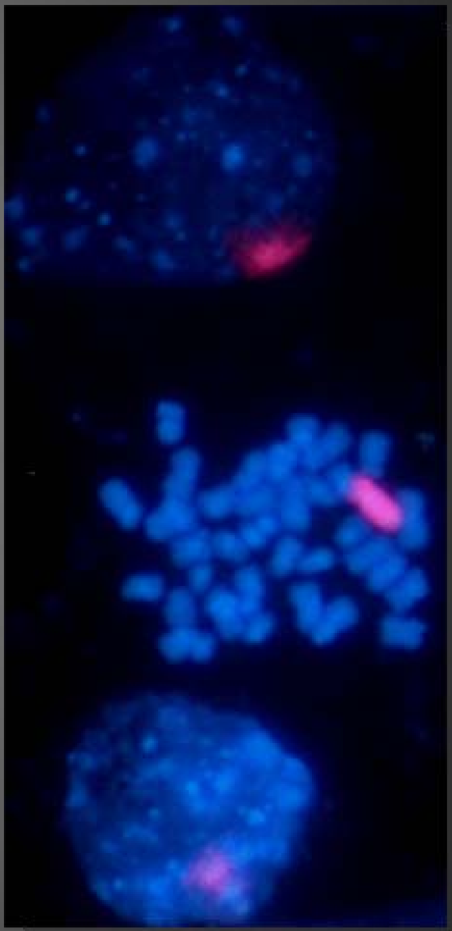
Pat Heslop-Harrison
Karine Alix
Xianhong Ge

Genome Specificity of a CACTA (*En/Spm*) Transposon

Brassica synthetic hexaploids

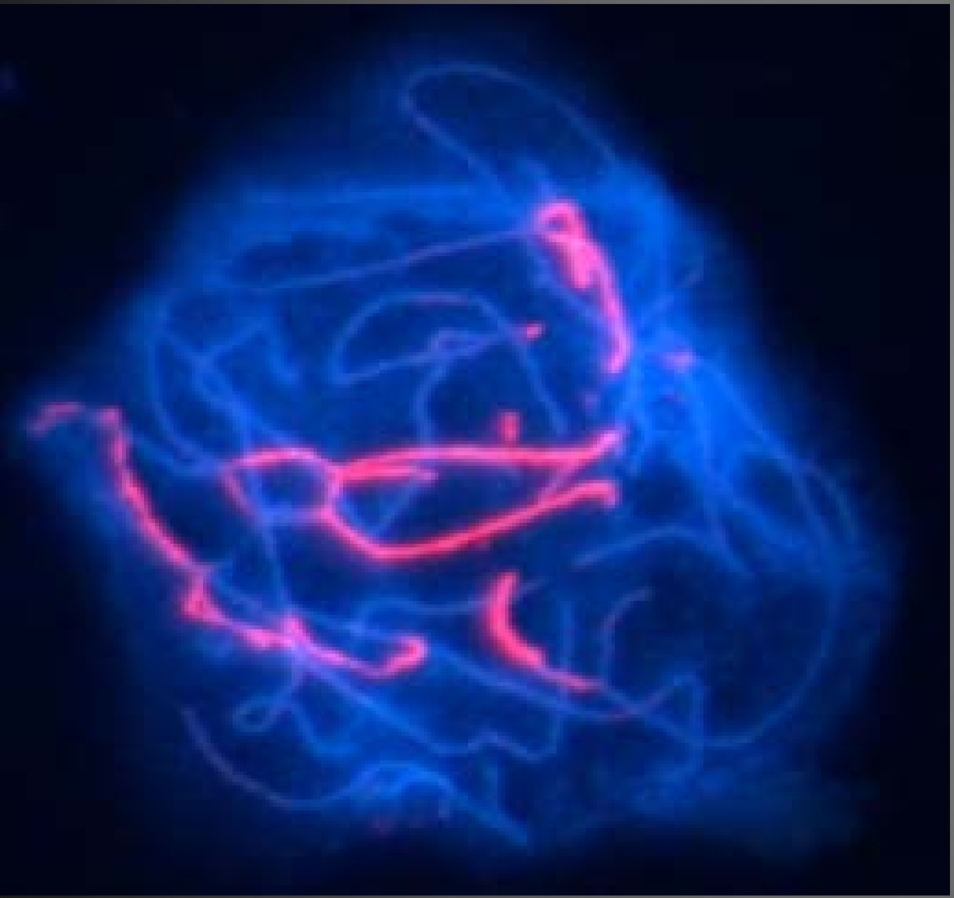
Brassica napus addition line *Orychophragmus violaceus*





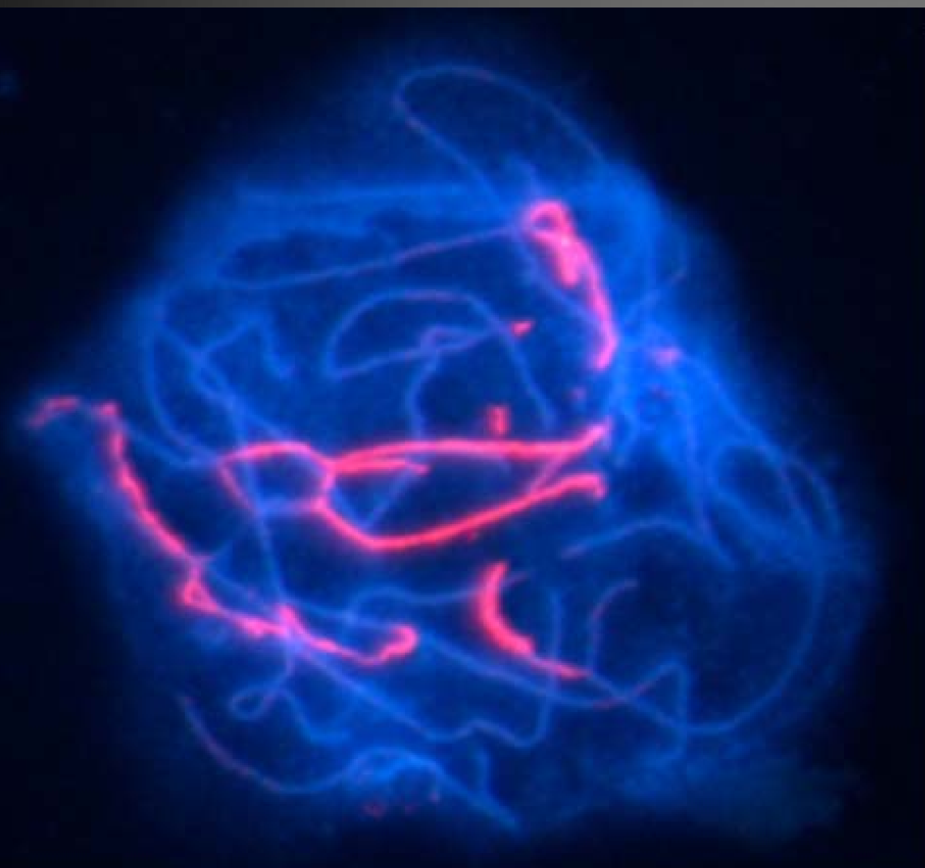
Xianghong Ge, Farah Badakshi, Heslop-Harrison and Schwarzacher 2010

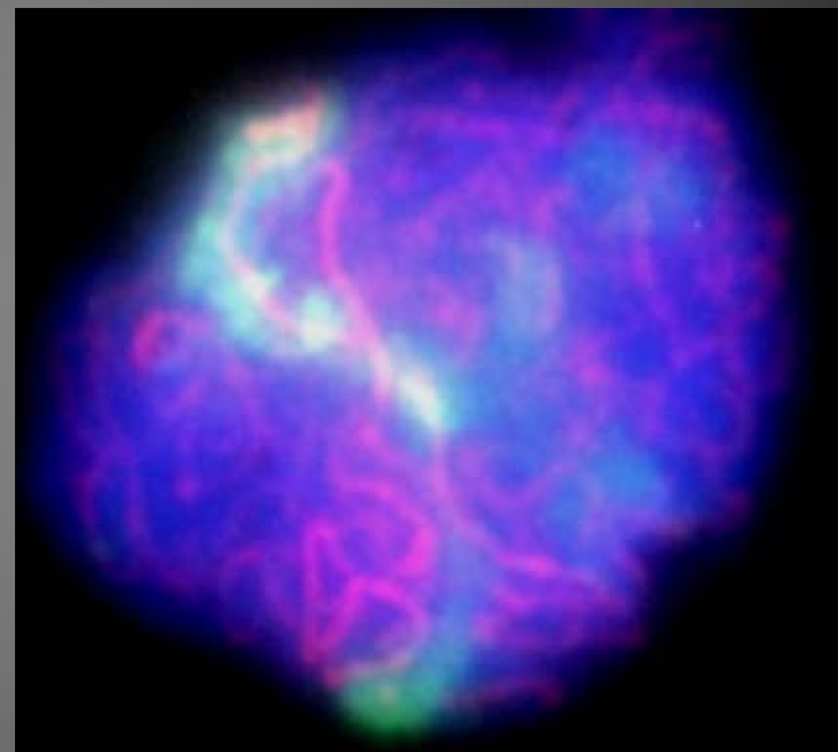
Brassica Monosomic Addition Line *Orychophragmus violaceus*



DAPI, ASY-1

Brassica Monosomic Addition Line *Orychophragmus violaceus*



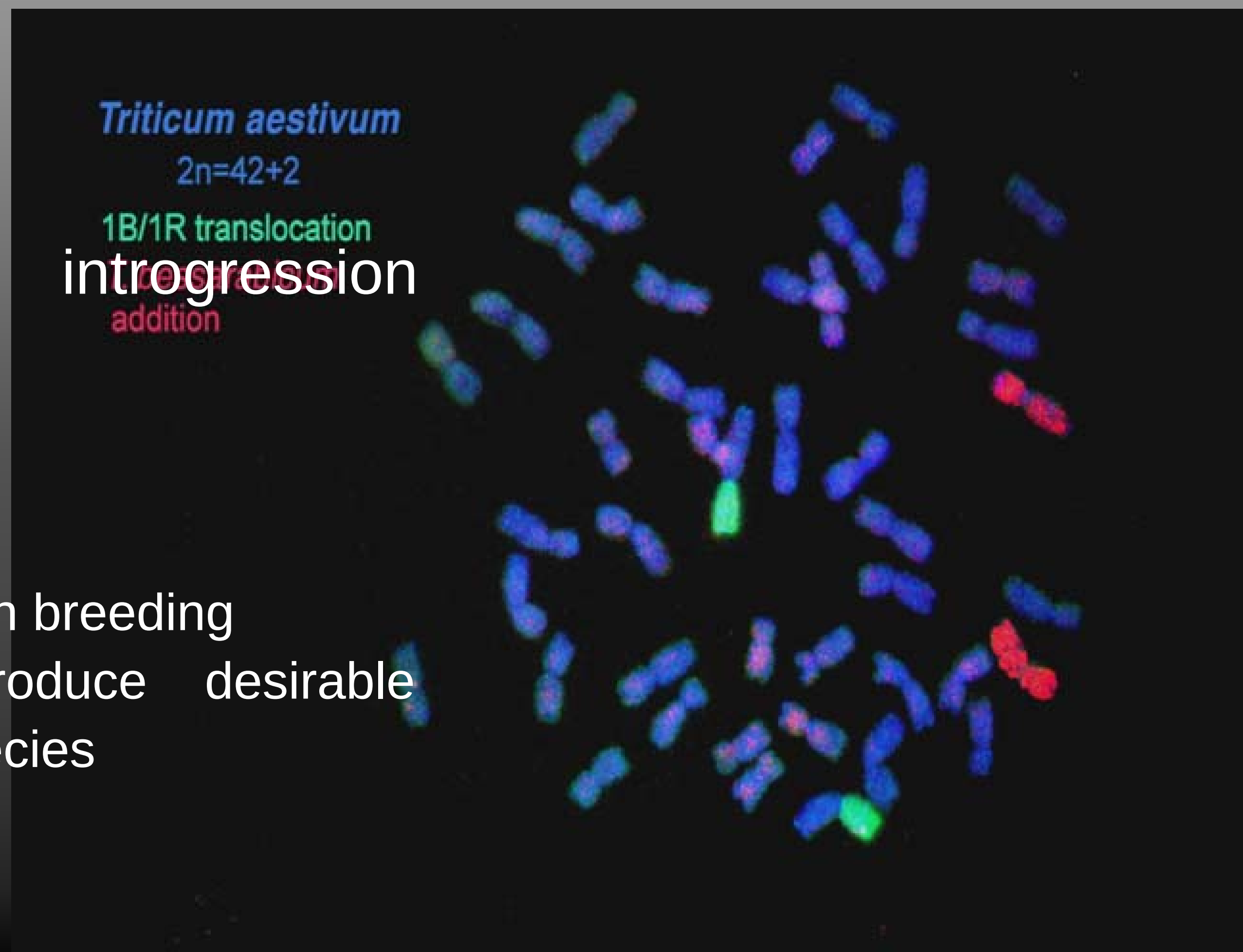


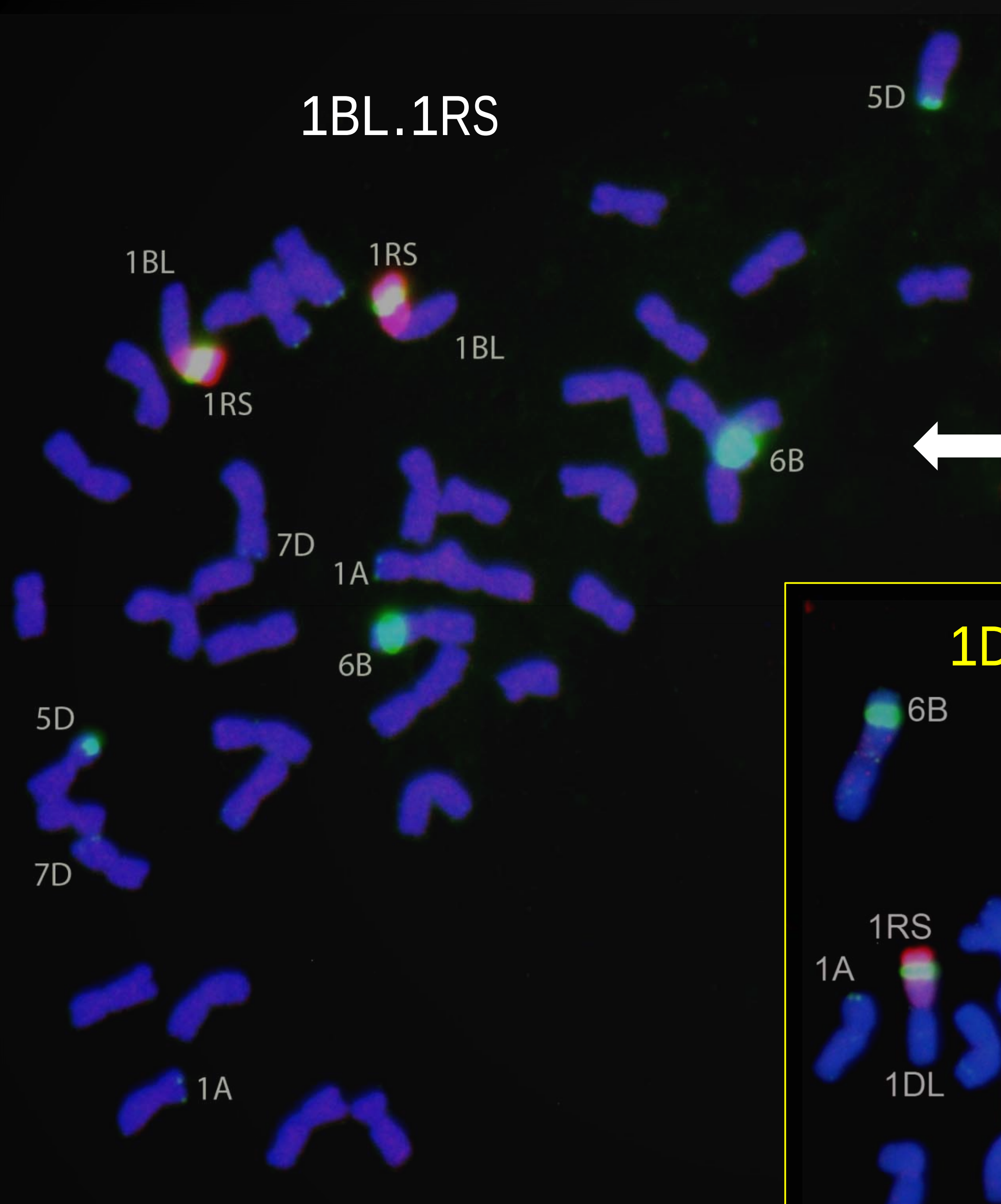
DAPI, ASY-1 *O. violaceus* genomic DNA
partly unpaired and partly paired with Brassica chromosomes

Alien gene transfer, alien recombinant chromosomes

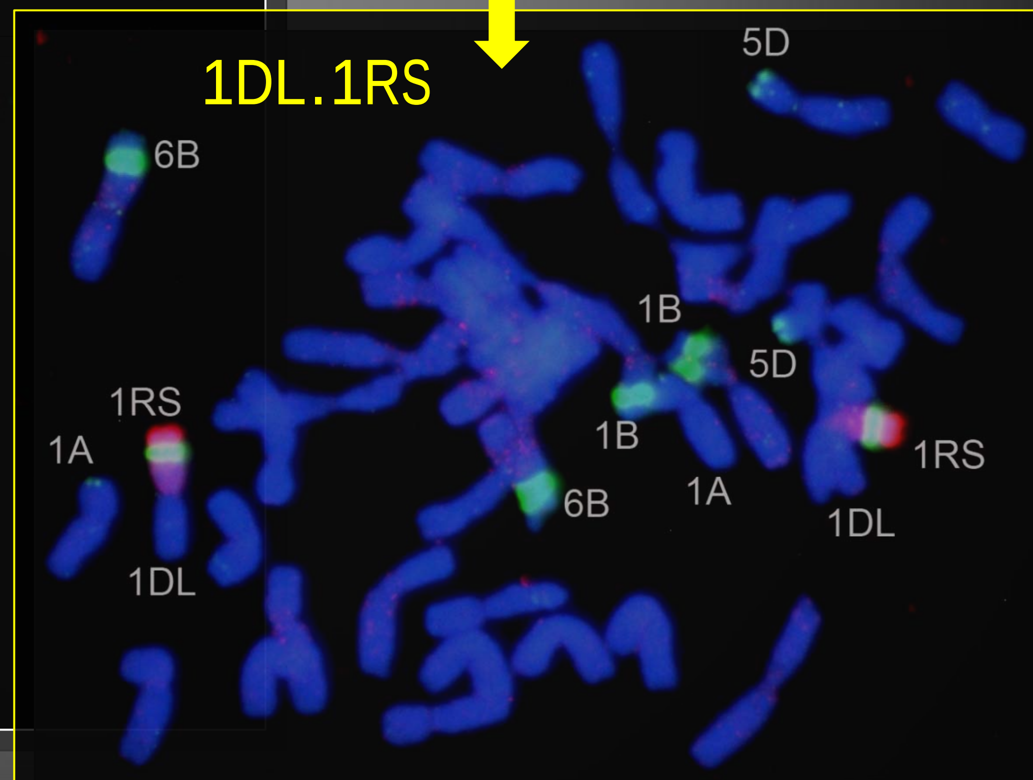
Total genomic DNA can be used as a probe to distinguish

- Genomes in sexual hybrids
- Alien chromosome introgression
    - Additions
    - Translocations
    - Used extensively in breeding programmes to introduce desirable traits from wild species



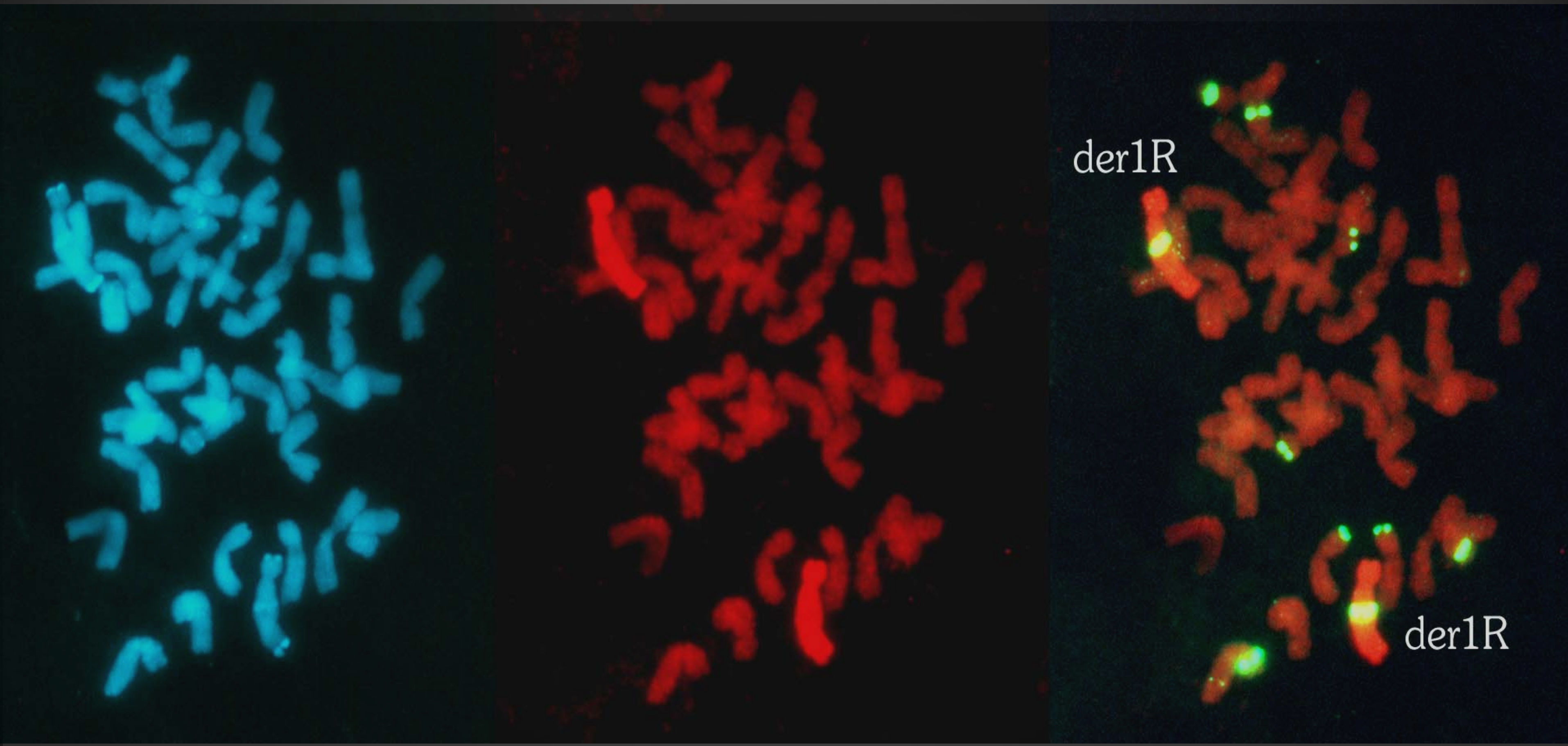


Rye DNA
pTa71-45S rDNA
4 major sites 1RS, 6BS
6 major sites 1RS, 1BS, 6BS



LINE 7-102 DERIVED FROM A TRITICALE X WHEAT CROSS

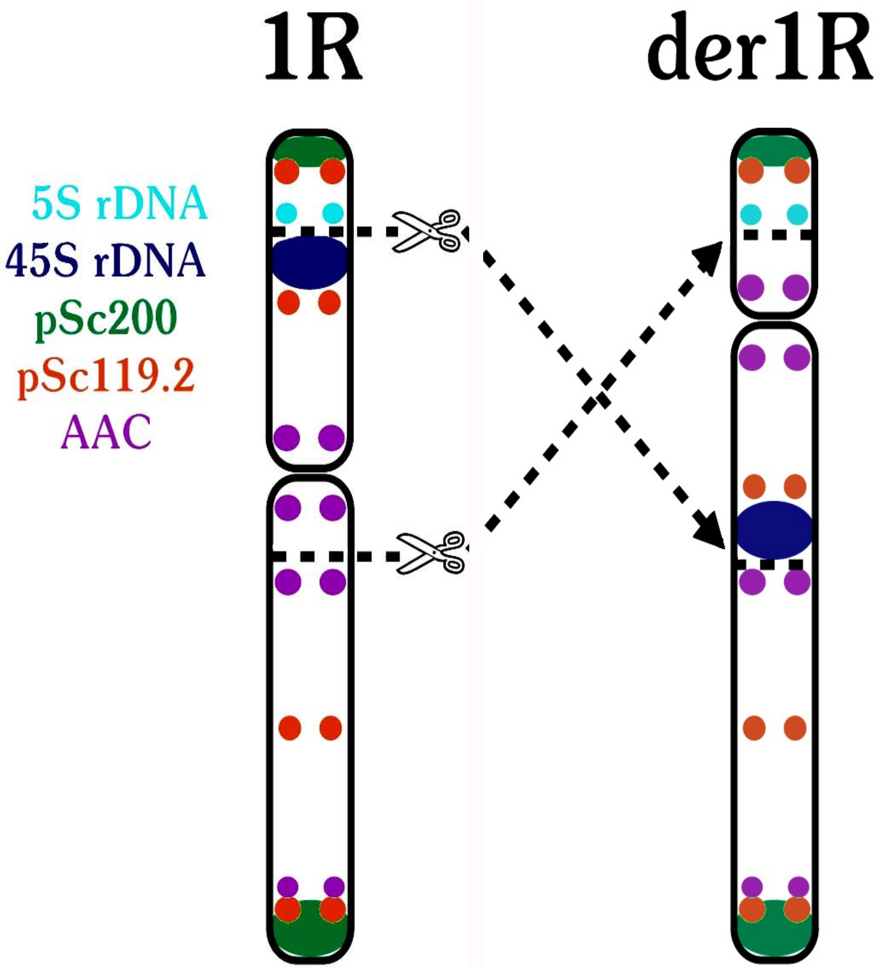
rye chromosome derivative 1R substitutes wheat chromosome 1D

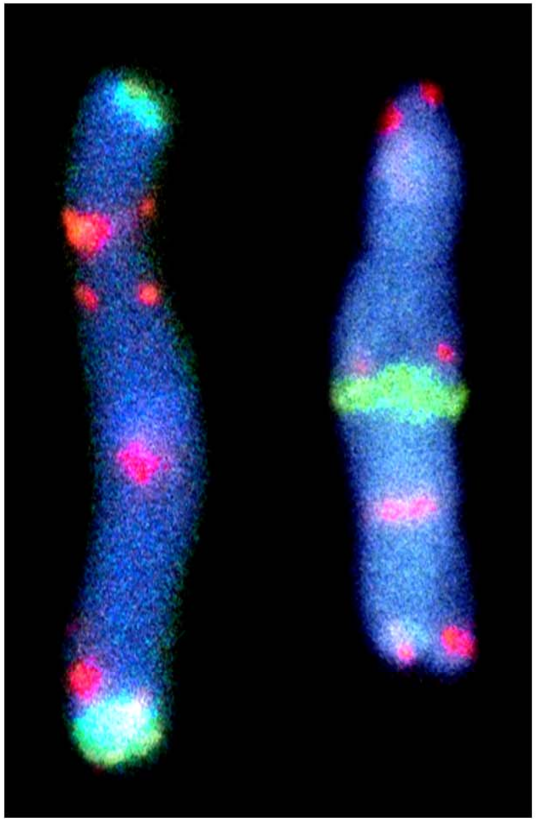


DAPI   Rye genomic DNA probe   pTa71 (45s rDNA probe)

Forsström and Schwarzacher 2000

Derivative chromosome 1R of Lines 7-102 and 7-169



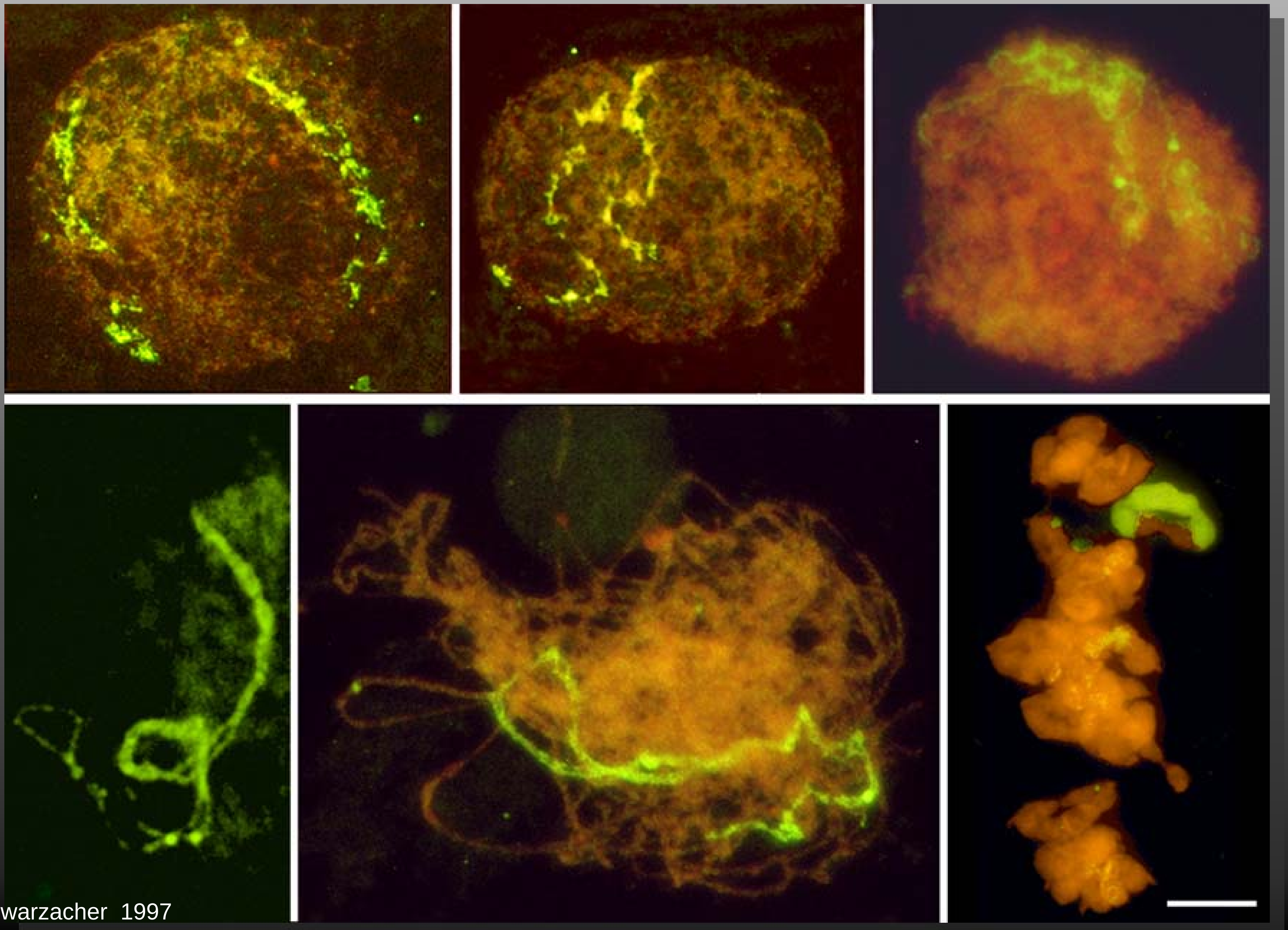


AAC
pSc200

pSc119.2
pTa71

Forsstrom and Schwarzacher 2000

Wheat 5AS.5RL at meiosis



Schwarzacher 1997

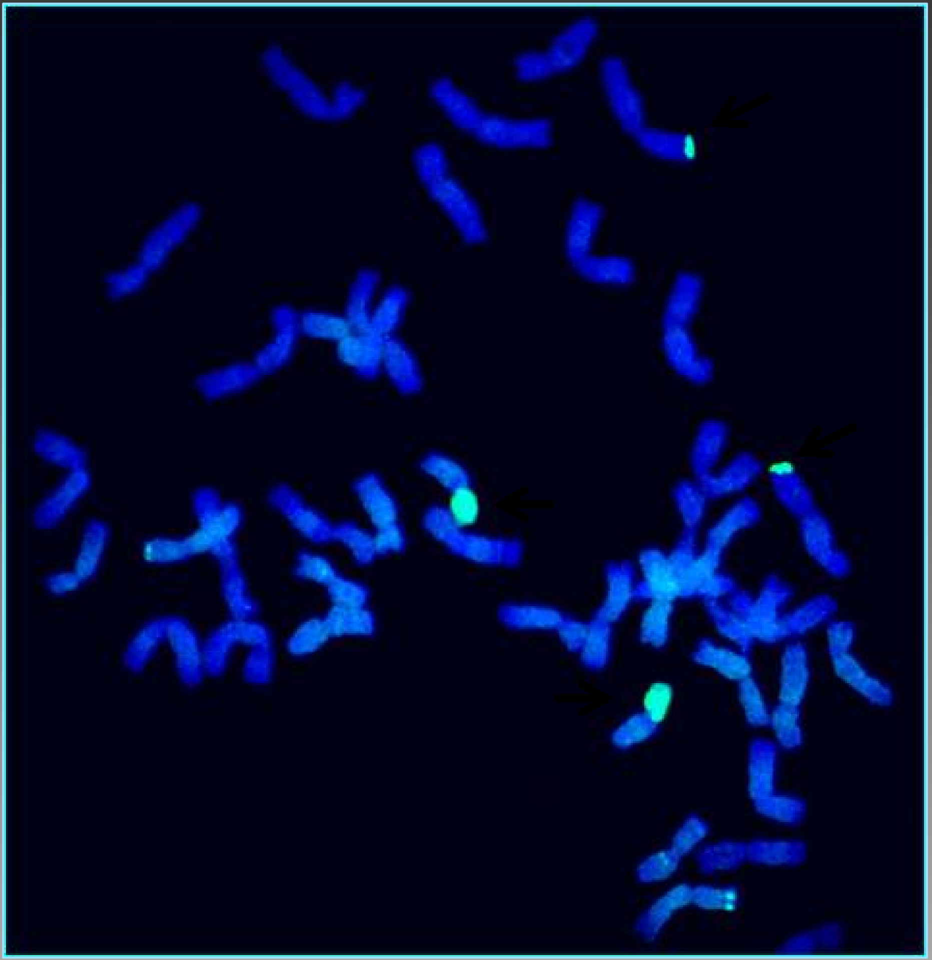
Characterization of new sources of Wheat streak mosaic virus resistance

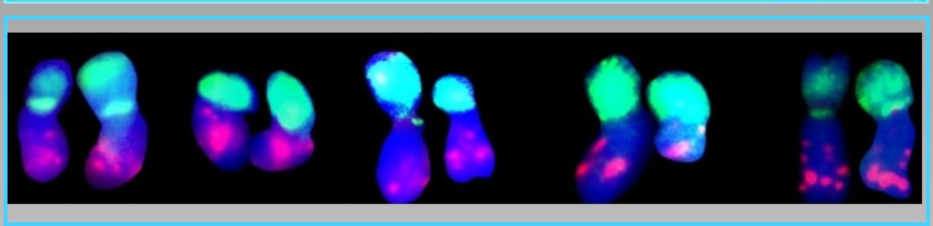
WSMV resistant and susceptible lines in field trials

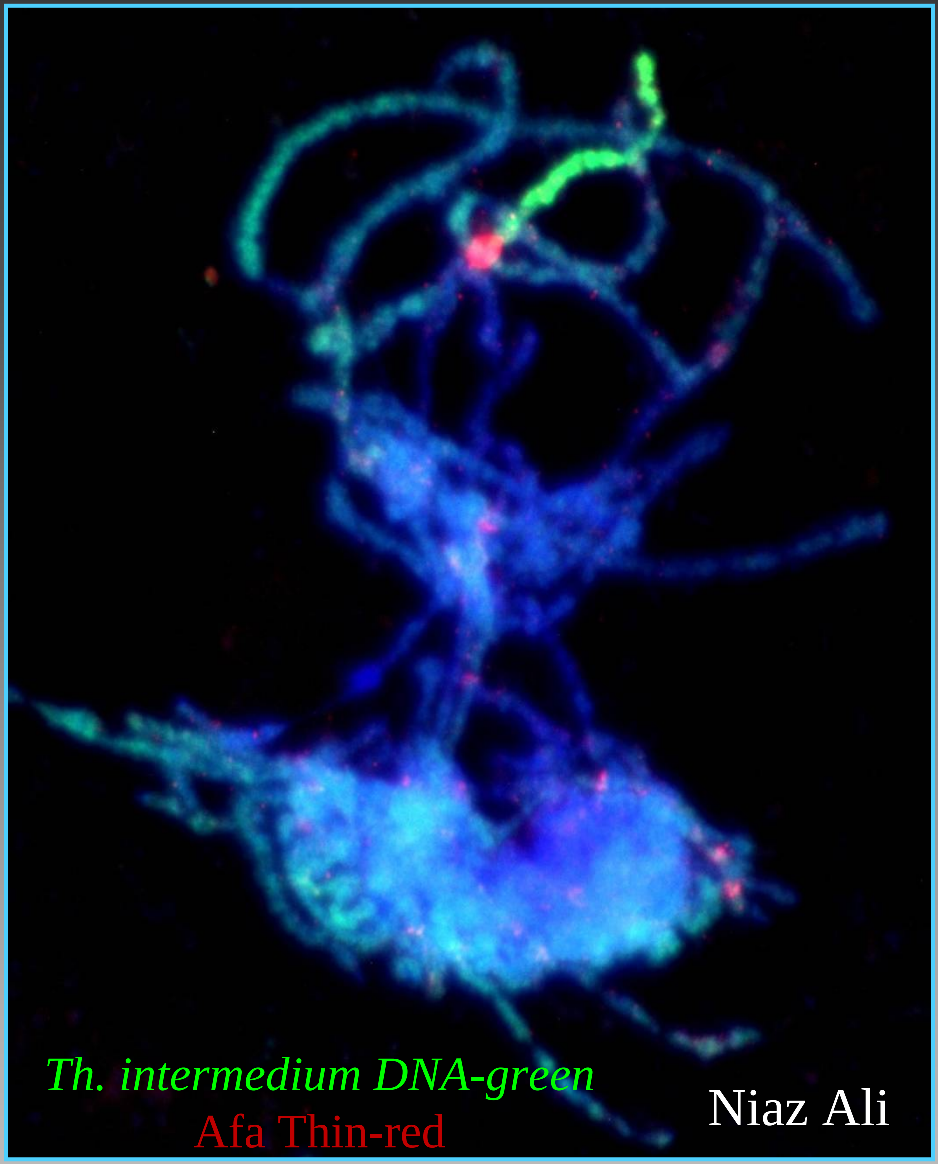
24 resistant and non-resistant sister lines from six different populations of wheat lines that potentially include an alien chromosome arm from *Thinopyrum intermedium* carrying WSMV resistance (Wsm-1 gene)

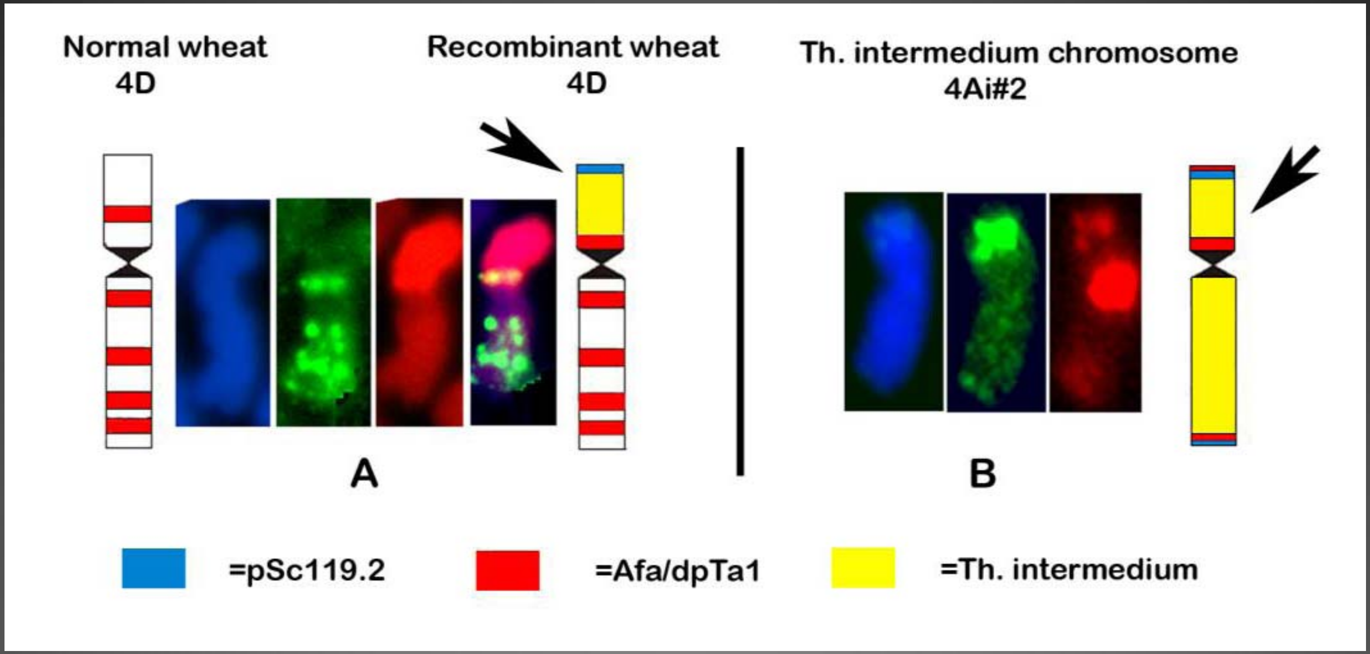
Bob Graybosch, USDA-ARS, University of Nebraska, USA

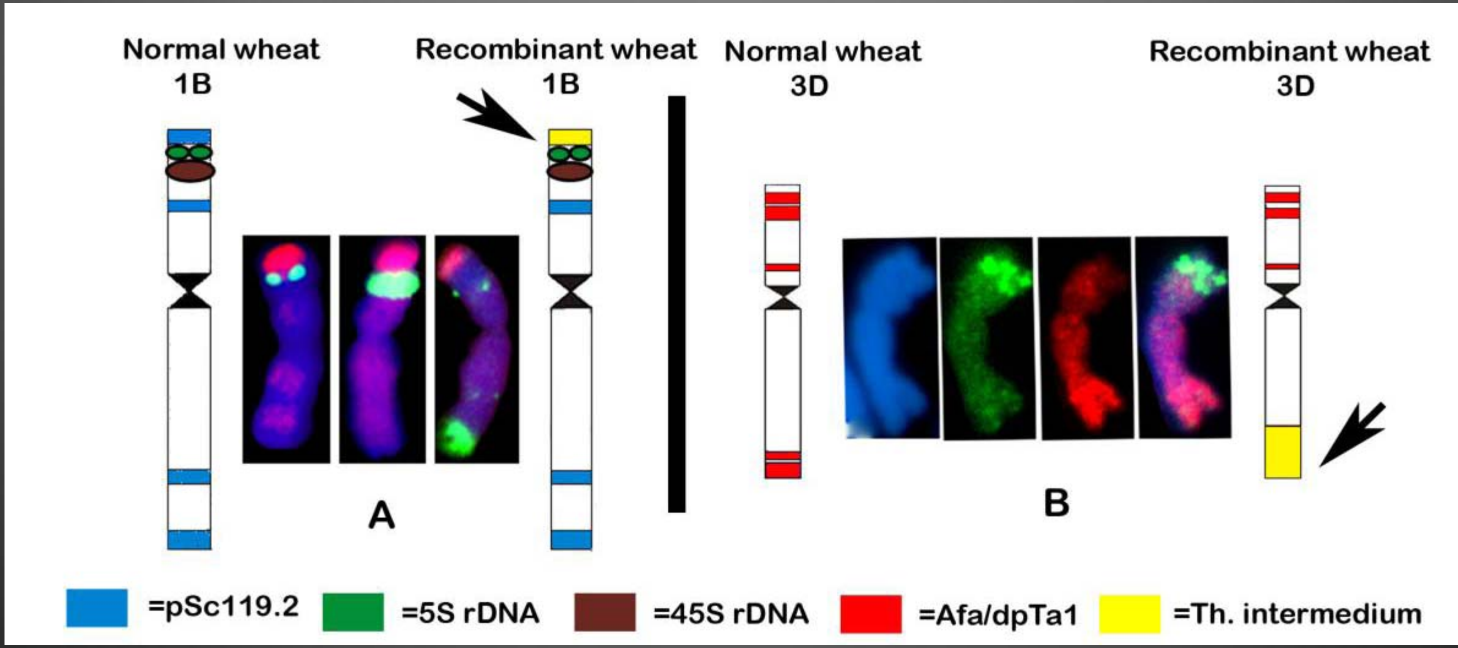
WHEAT WITH T4DL.4J^{S}S TRANSLOCATION:



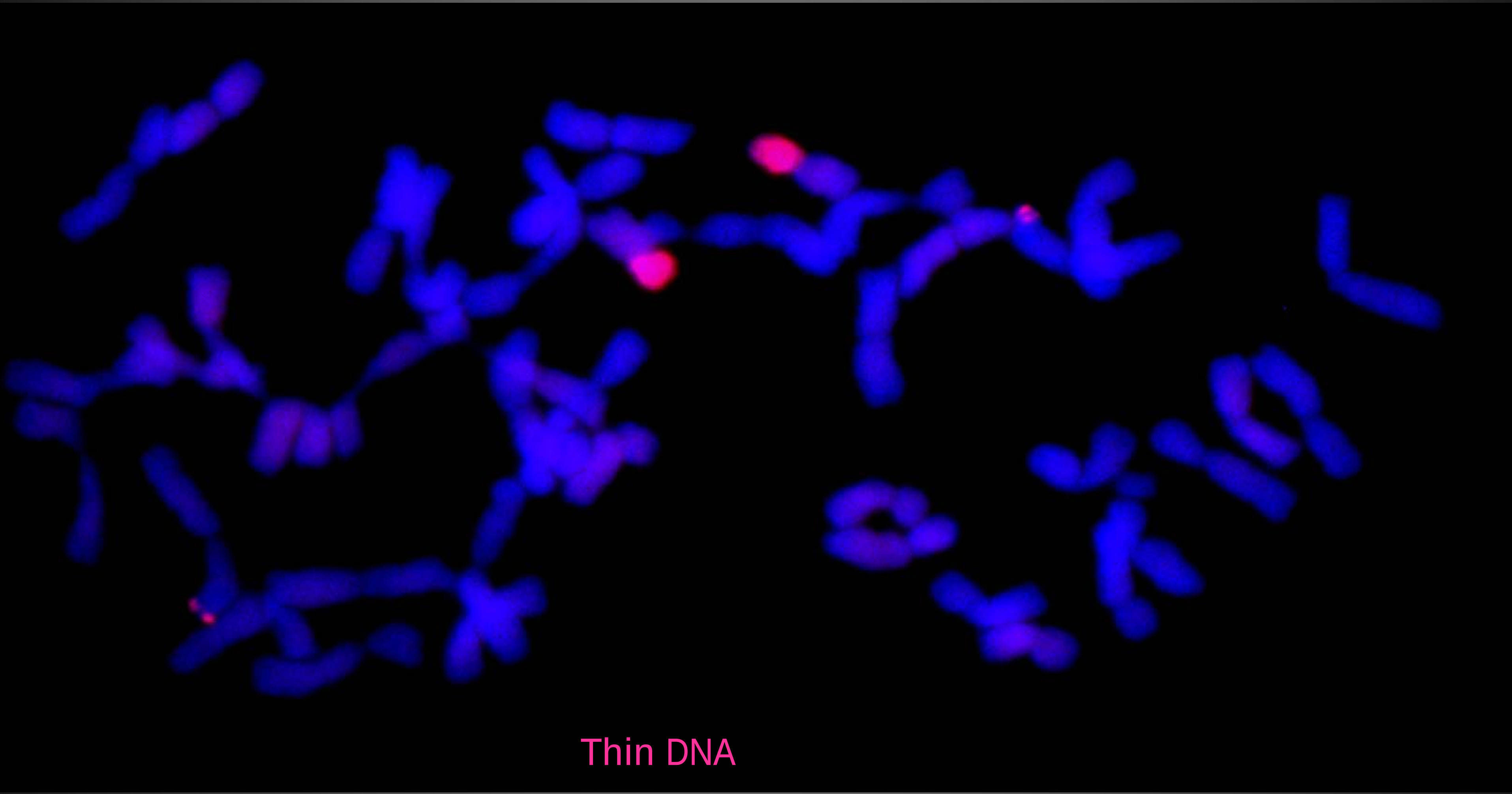






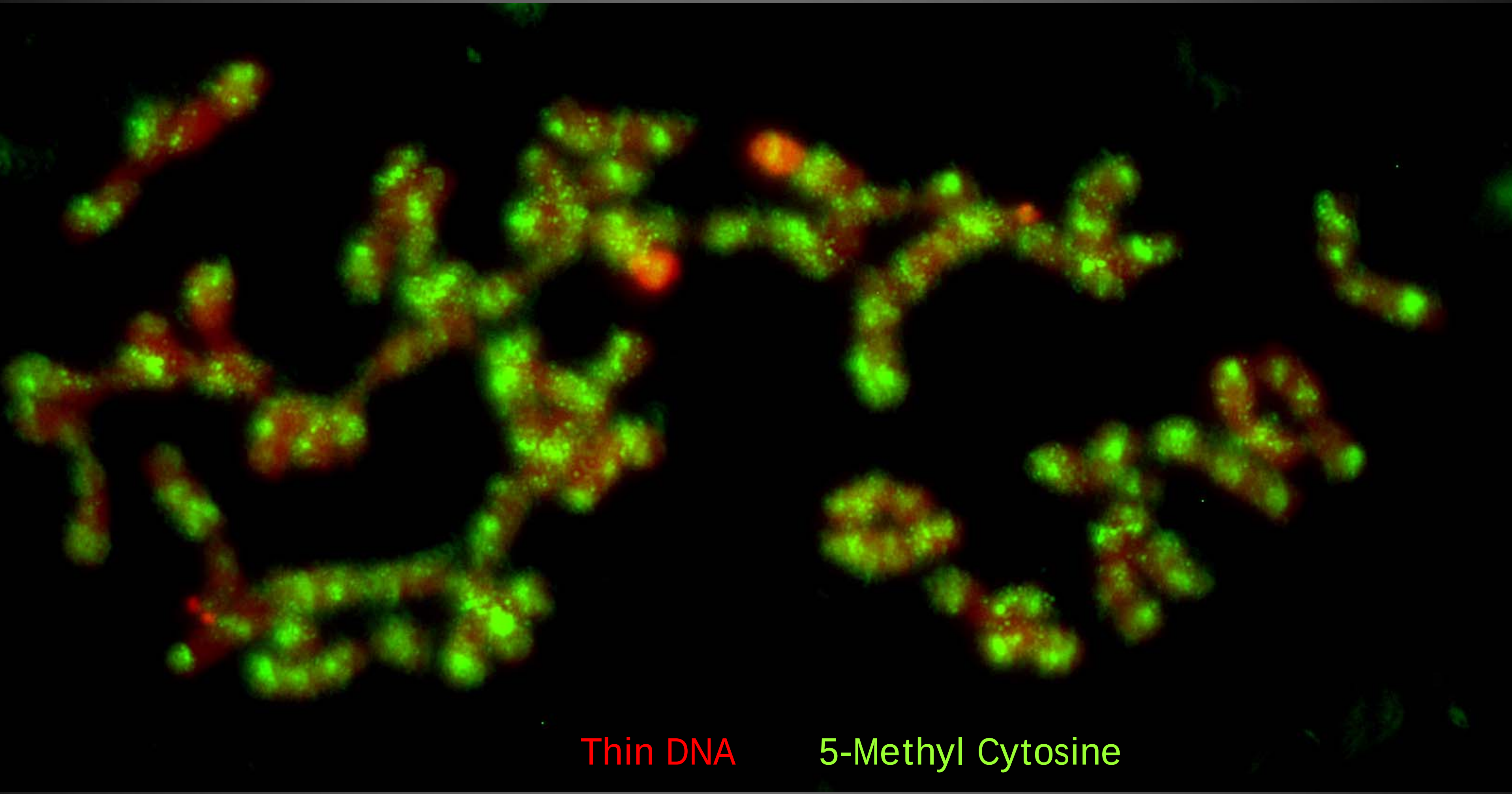


Niaz Ali, PhD thesis 2012



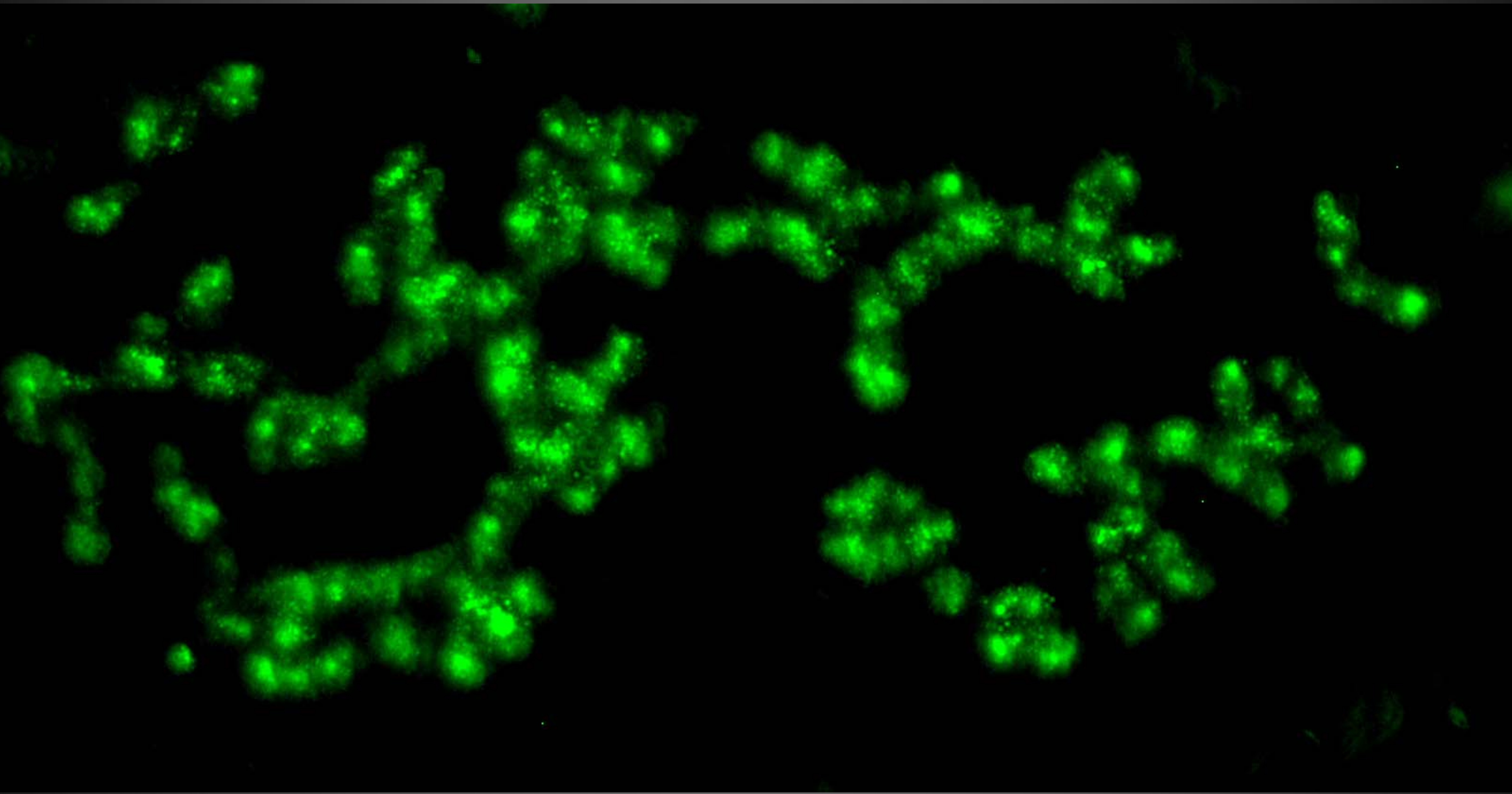
Niaz Ali

The alien Thin fragment is not heavily methylated



Niaz Ali

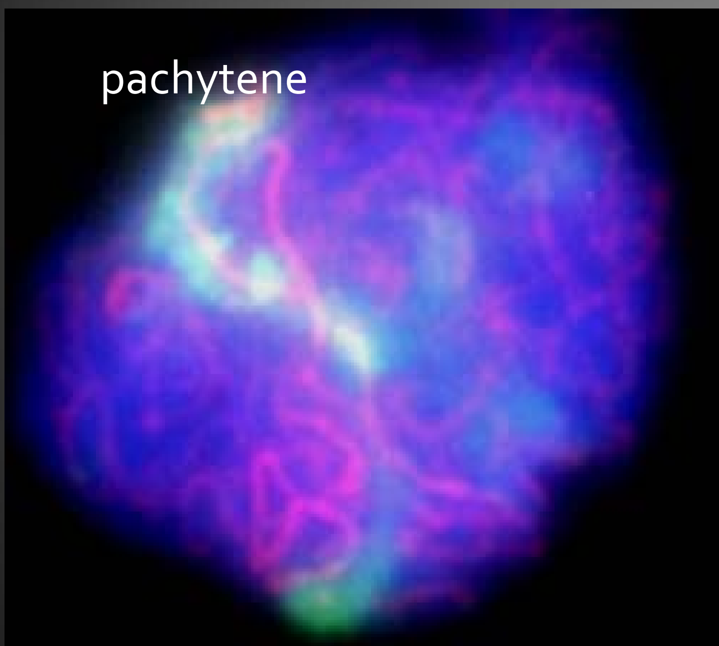
The alien Thin fragment is not heavily methylated



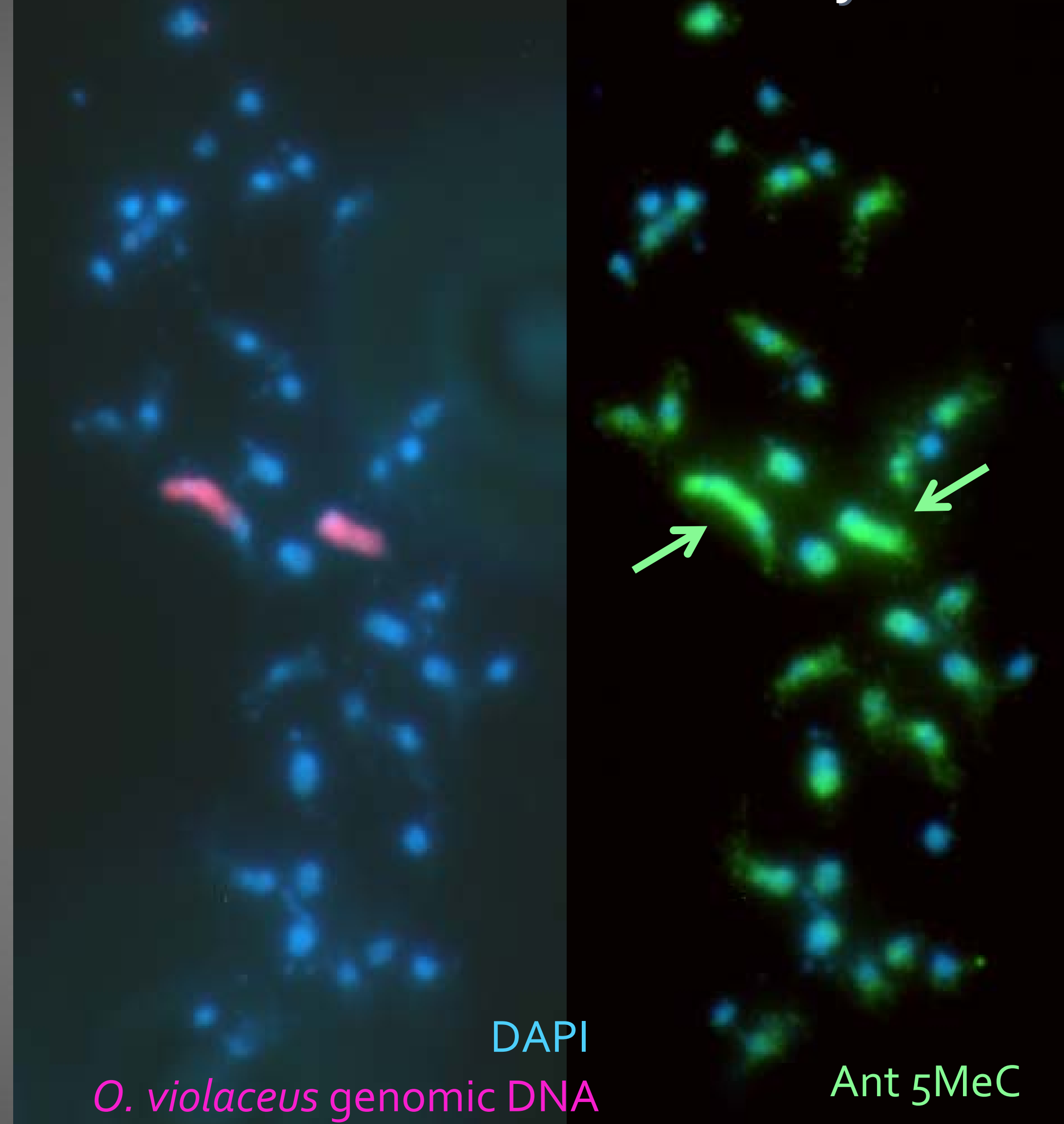
Niaz Ali

ADDITION LINE *ORYCHOPHRAGMUS VIOLACEUS*

Increased DNA methylation

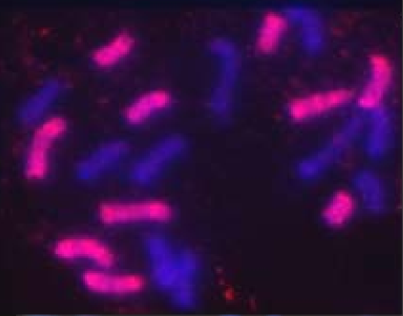
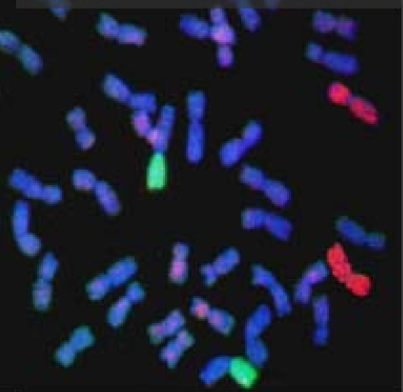
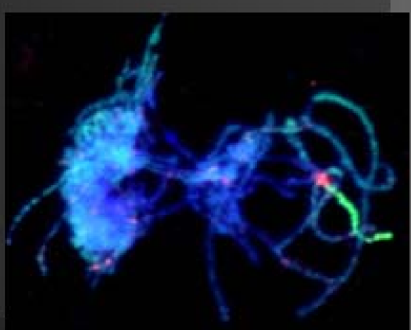
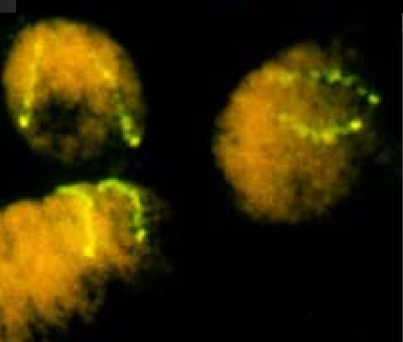


DAPI, ASY-1
O. violaceus genomic DNA
Homozygous partly unpaired and partly paired with Brassica chromosomes



Xianghong Ge, Farah Badakshi, Heslop-Harrison and Schwarzacher 2010

GENOMIC *IN SITU* HYBRIDIZATION GISH

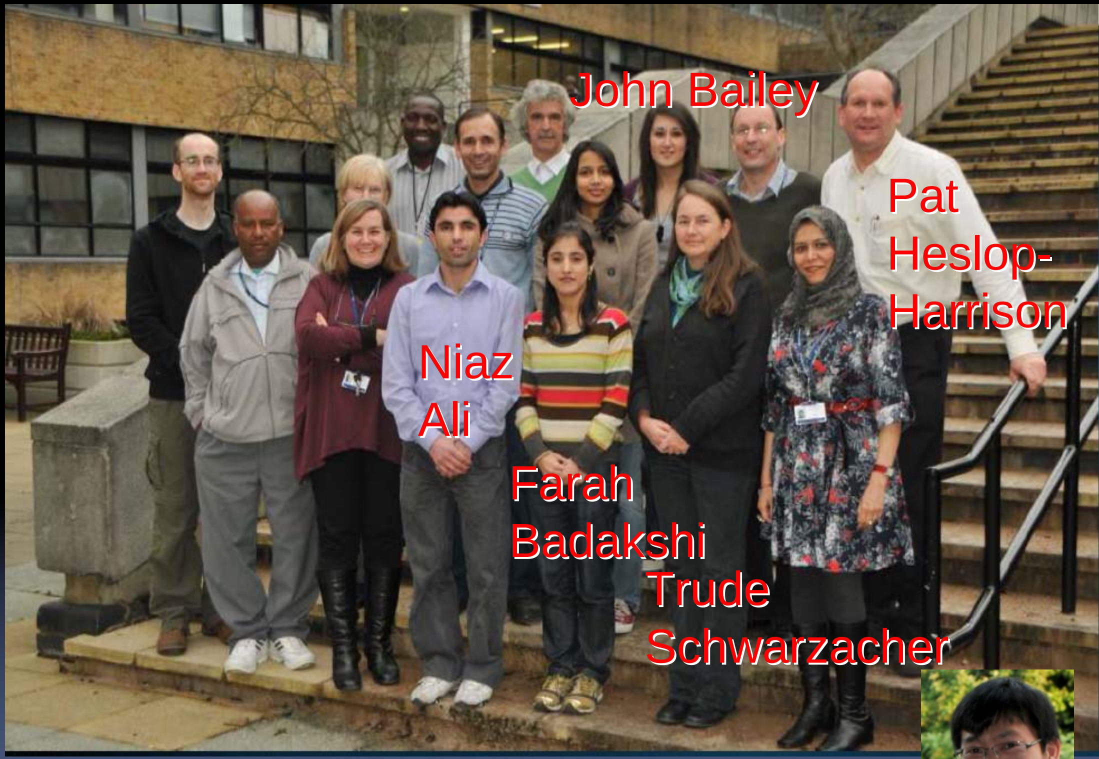





- Parental origin of hybrids
  - Auto or allo-poliploidy
- Alien chromatin in breeding lines
  - Size and origin
  - Recipient chromosome
- Meiosis and chromosome pairing
- Interphase cytogenetics

- Understanding hybrid genomes
  - Chromosome behaviour
  - Chromatin function

Thank you to members of the Molecular cytogenetics lab

- Collaborators and past members of the lab
  - Richard Gornall, Leicester
  - Bob Graybosch, Nebraska, USA
  - Karine Alix, INRA/Univ Paris-Sud, France



Xianhong Ge , Huazhong Agricultural University, Wuhan, China

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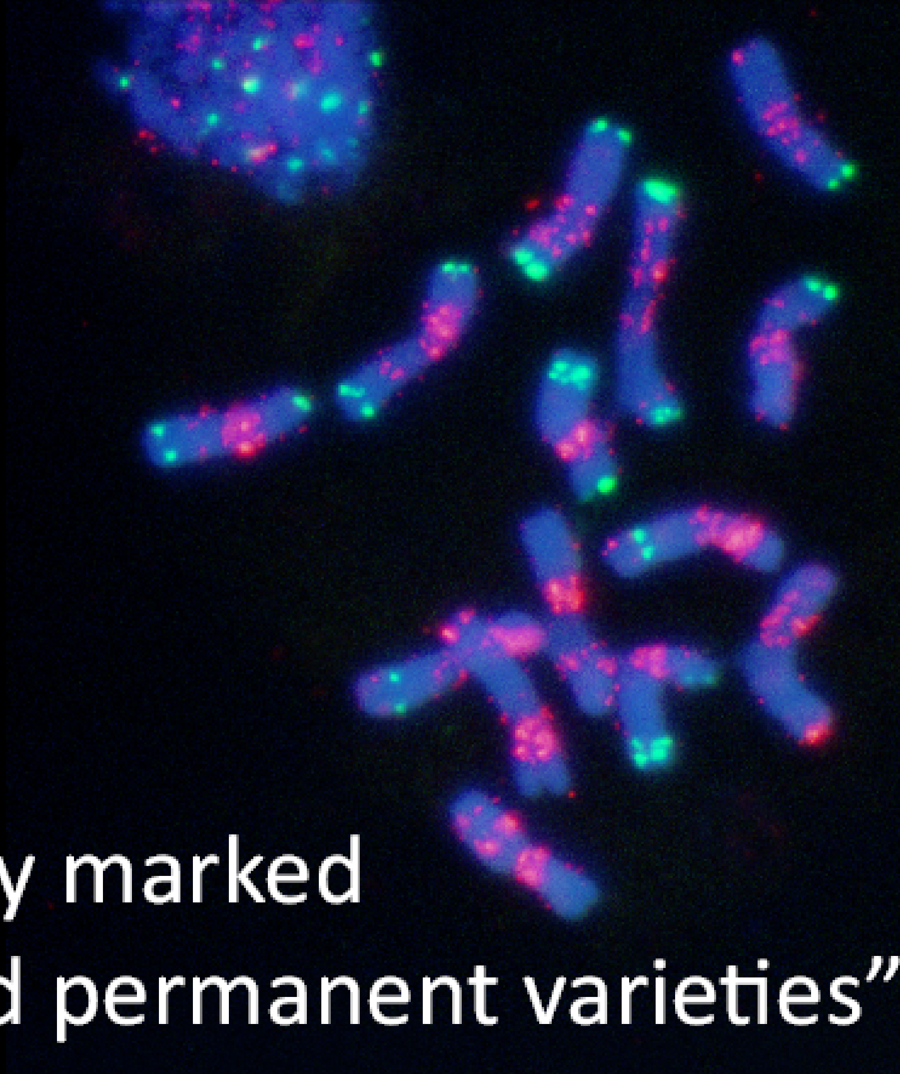
The Origin of Species begins with an example of how humans have domesticated certain species and used artificial selection, in contrast to natural selection, to produce extraoridnary variation in a short time. Darwin focused on “fancy” pigeons, but it is apparent in many dopmestic species, including dogs, cattle and crop plants like wheat (see below).

At that time, it was not known how such variation arose or was maintained. Now we know that this variation is due to genes and chromosomes, and a team here in Leicester investigate how the number and organisation of chromosomes varies across wheat varieties.

Dr Trude Schwarzacher and Prof Pat Heslop-Harrison investigate the evolution of cereals by examining their chromosomes. On the right are the chromosomes from a root cell of rye. The red and green spots are DNA sequences which can be used to trace the ancestry of different cereal species and varieties.



“Species are only strongly marked and permanent varieties”

Below are examples of wheat ears from different hybrids and wild varieties of wheat.