

Chromosome Analysis and Molecular Cytogenetics

A fluorescence micrograph of a chromosome spread. The chromosomes are stained with a blue dye, likely DAPI, to visualize their structure. Several chromosomes show bright green fluorescence, indicating the presence of specific DNA sequences. A few chromosomes also show bright red fluorescence, likely representing a different marker or probe. The background is dark, making the fluorescent signals stand out.

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www.molcyt.com

Including talk slides!

www.molecularcytogenetics.com

UserID/PW 'visitor'

Blog: AoBBlog.com Twitter: [pathh1](https://twitter.com/pathh1)

March 2011

Crocus species and hybrids



C. flavus

$2n=8$



C. 'Stellaris'

$2n=2x=10$



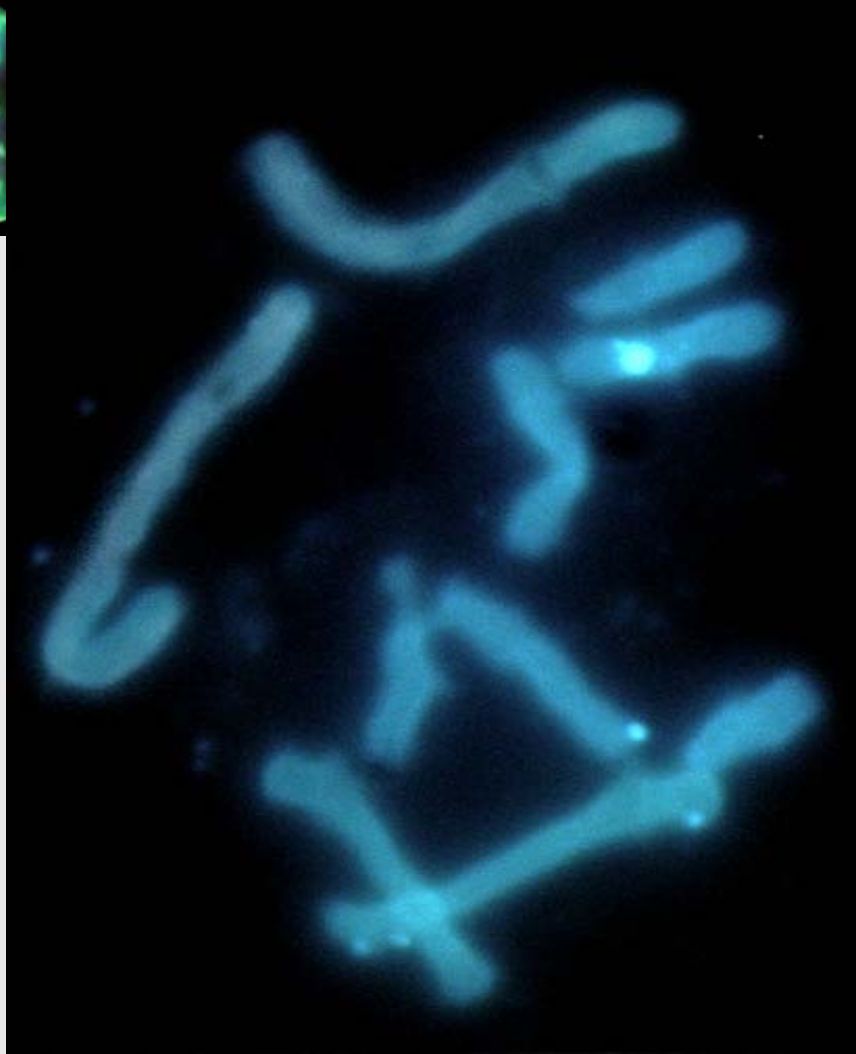
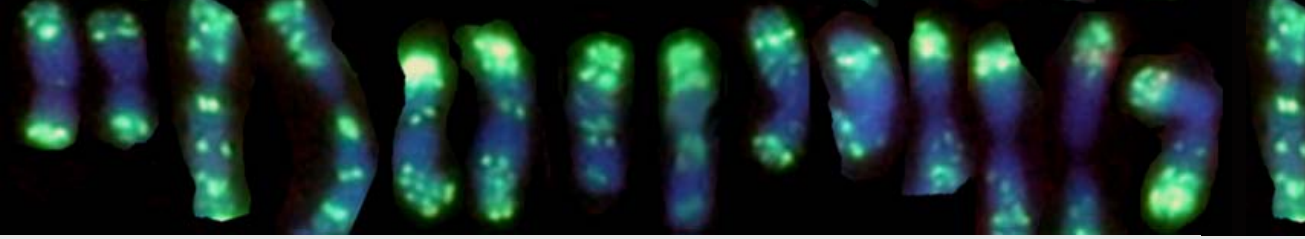
C. angustifolius

$2n=12$

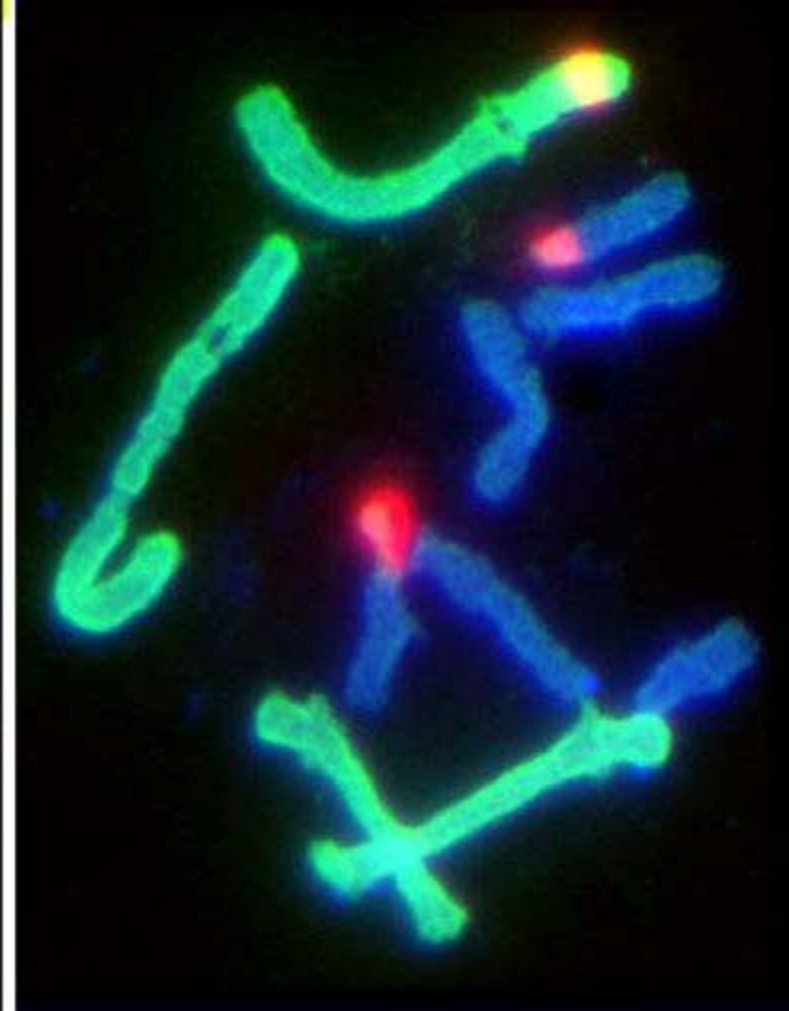
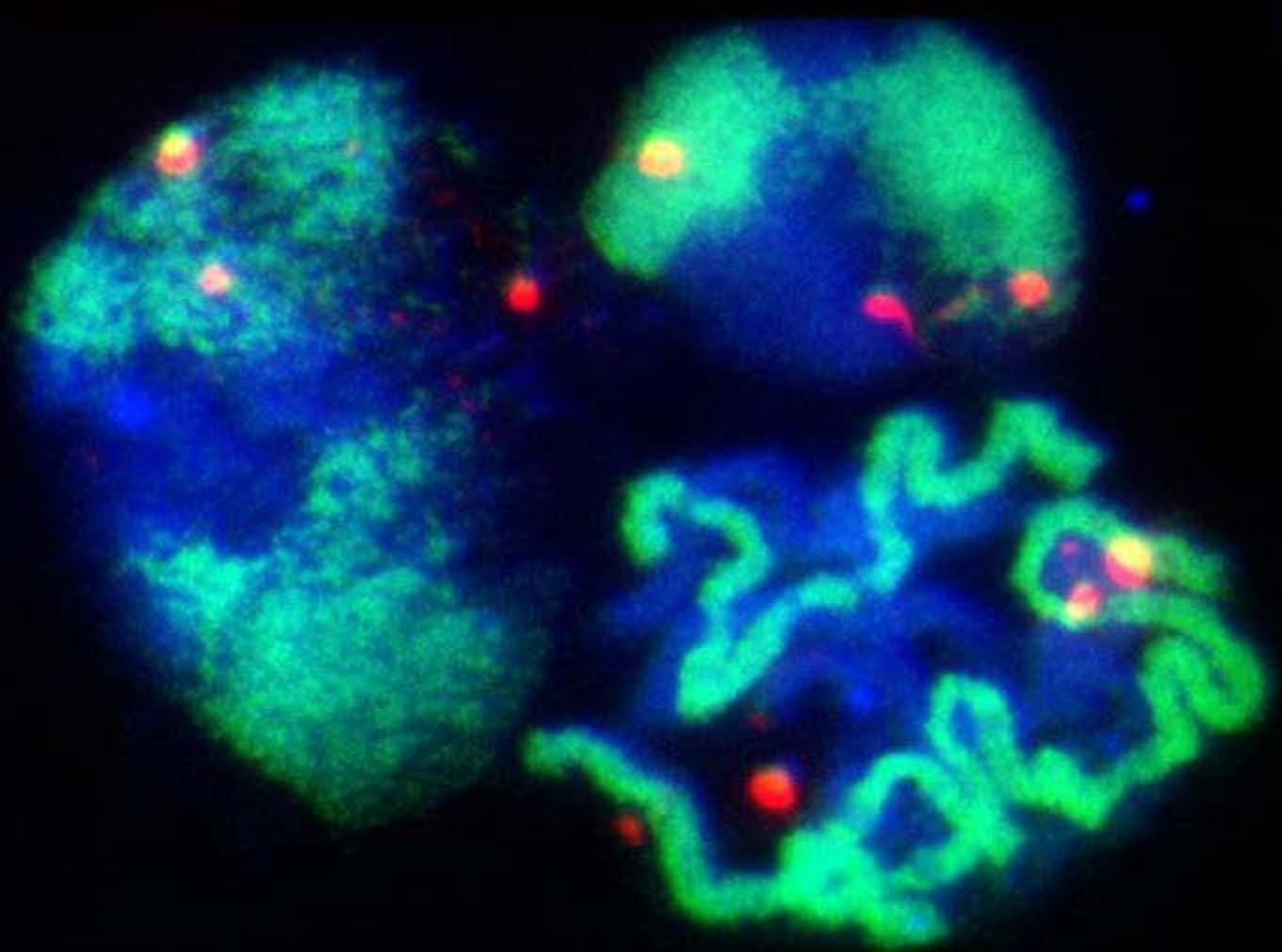


C. 'Golden Yellow'

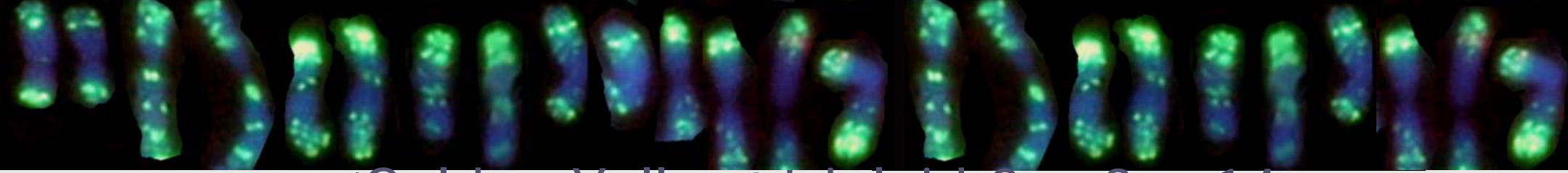
$2n=3x=14$



- The parents look similar, sharing many genes
- Total genomic DNA as a probe labels the parental genomes differentially



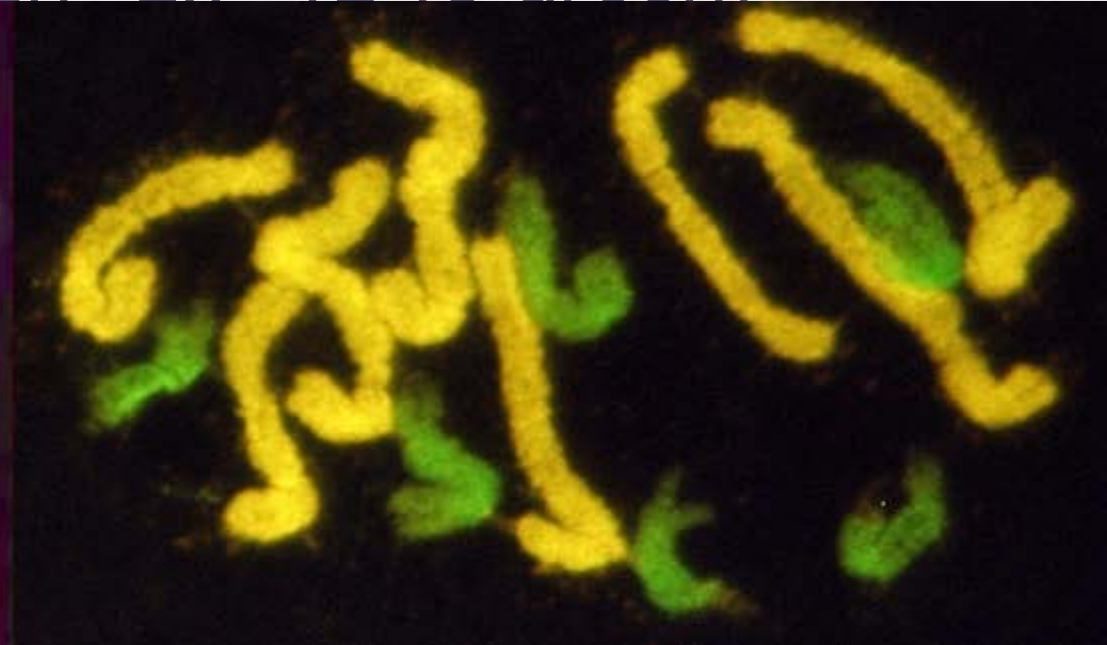
- ☛ The parents look similar, sharing many genes
- ☛ Total genomic DNA as a probe labels the parental genomes differentially
- ☛ Farah Badakshi, John Bailey, Trude Schwarzacher, Marian Orgaard



'Golden Yellow' triploid $2n=3x=14$

C. flavus $2n=2x=8$ (8 yellow)

C. angustifolius $2n=2x=12$ (6 green)



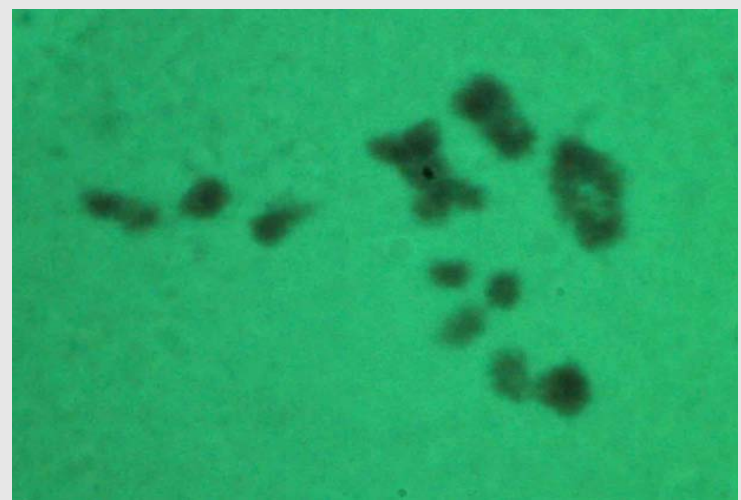
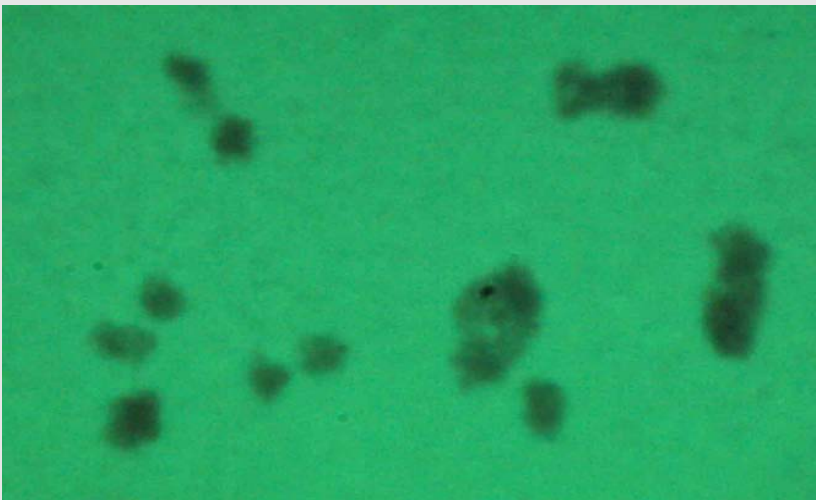
Orgaard, Jacobsen & HH

Metaphase I in triploid Golden Yellow Crocus



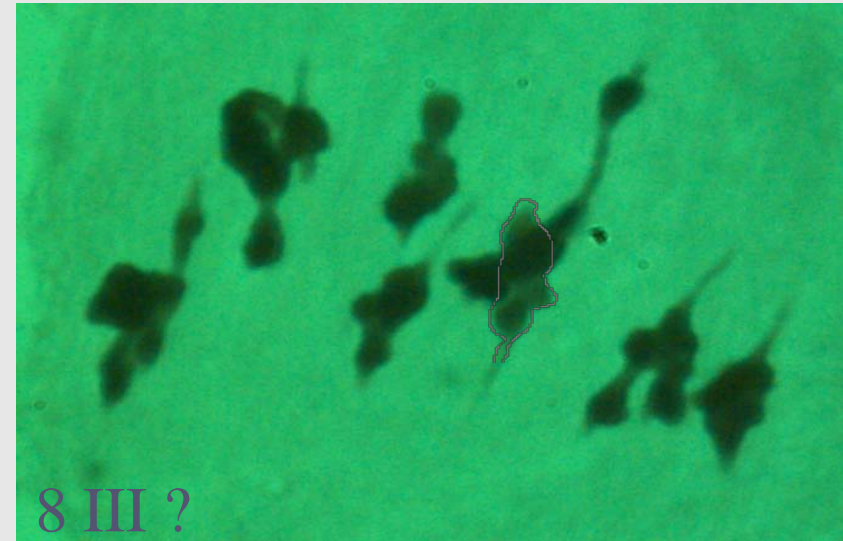
In many metaphase I cells of this triploid, we see four bivalents from pairing of the four pairs of *C. flavus* ($2n=2x=8$)-origin chromosomes, with the six chromosomes from *C. angustifolius* ($2n=2x=12$) present as univalents.

Alternative meiotic configurations were also identified: eg 3 bivalents and 8 univalents.

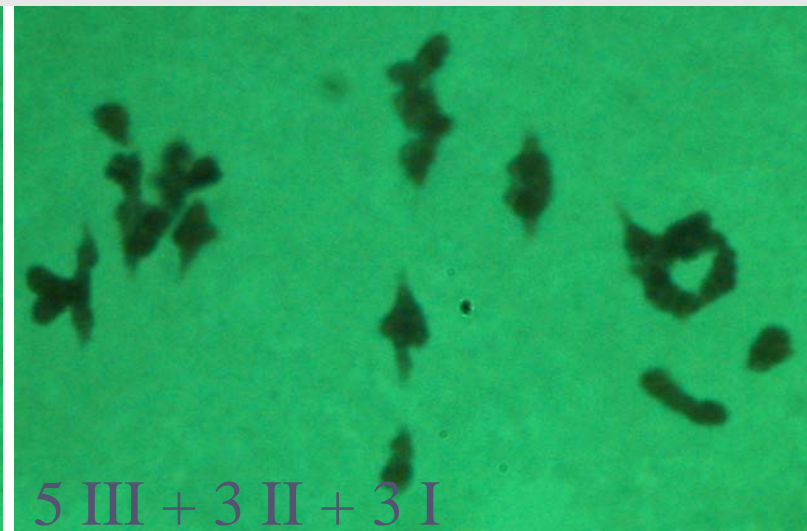
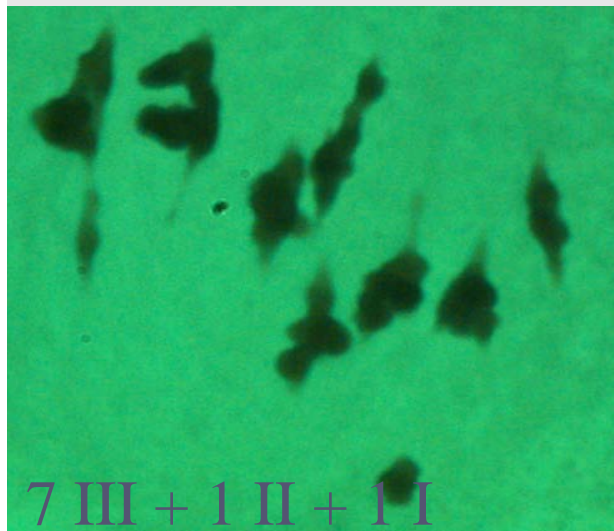
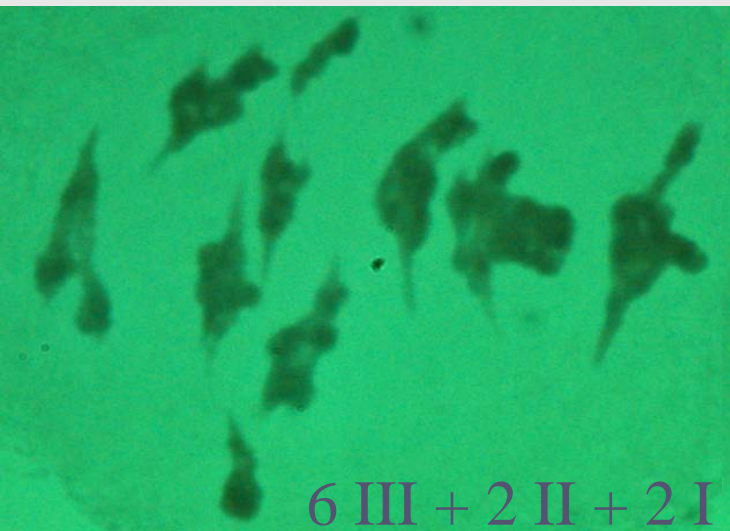


Metaphase I In triploid Saffron (*C. sativus*)

Saffron, the world's most expensive spice, consists of the stigmas of *Crocus sativus*, a sterile triploid ($2n=3x=24$). One wild ancestor is *C. cartwrightianus* ($2n=2x=16$), but the other is unknown.

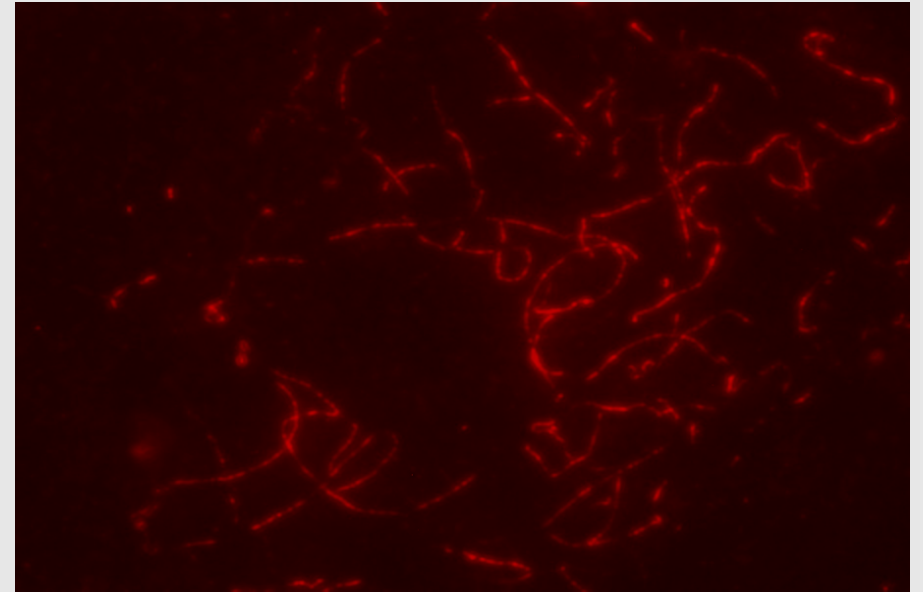
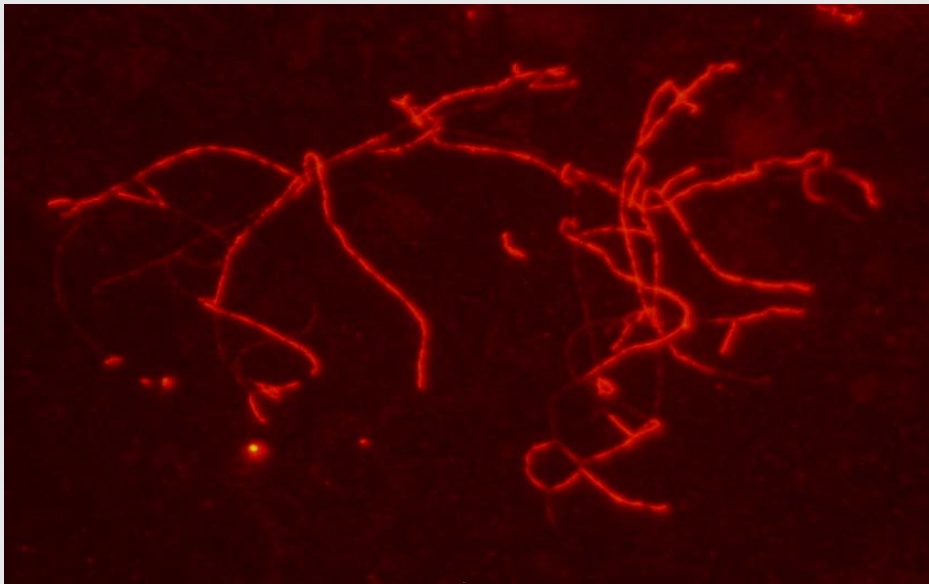
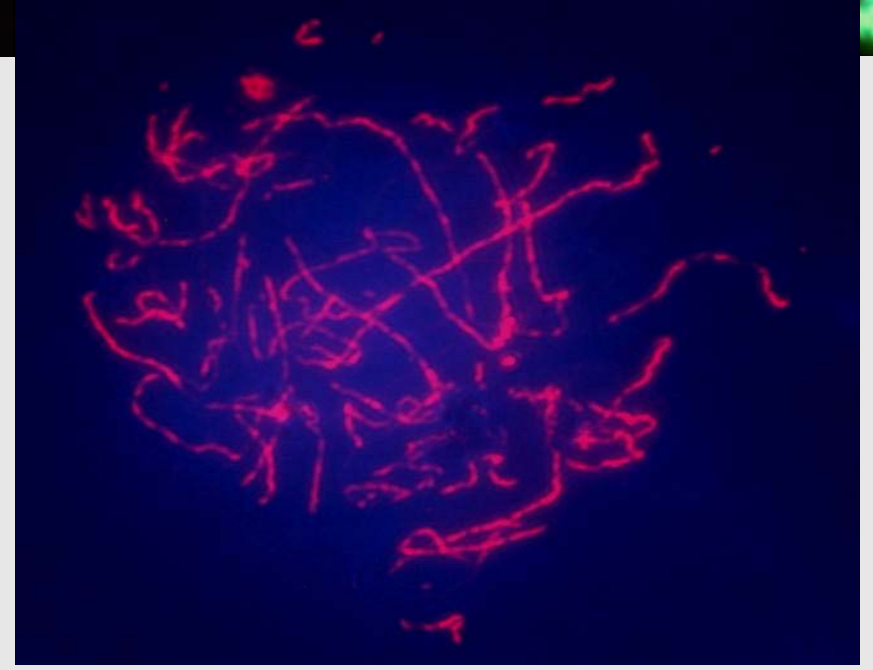
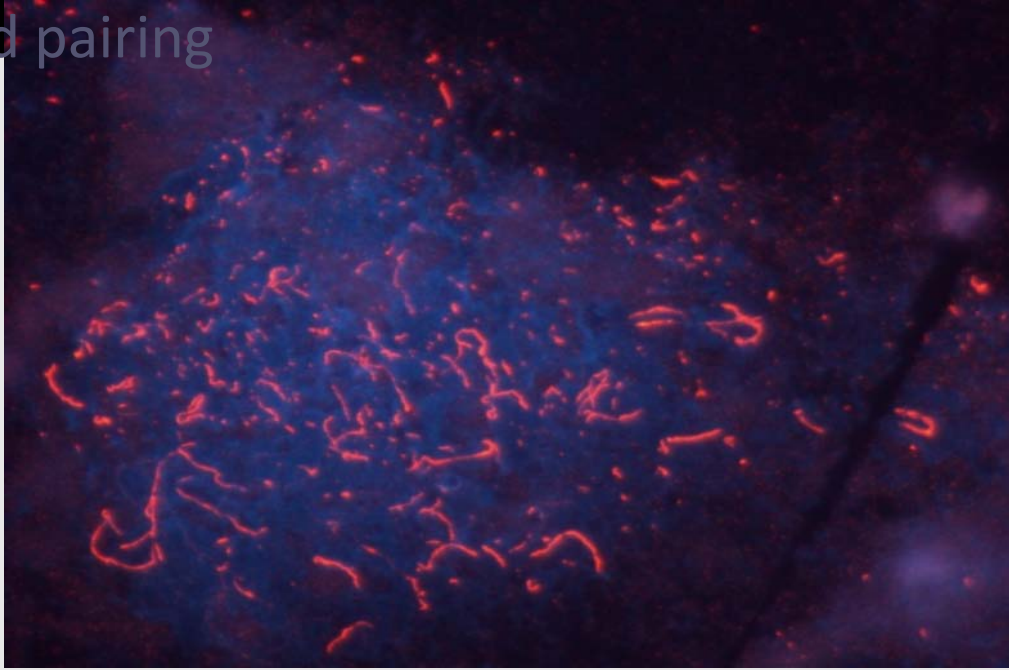


Metaphase I cells of this triploid ($2n=3x=24$) showed as few as 8 structures, a mixture of trivalents, quadrivalents and univalents.



Meiotic prophase in triploid Saffron (*C. sativus*)

$\Delta S4-1$ and $\Delta 600$ ES (Franklin and Armstrong) show the progression of lateral element formation and pairing

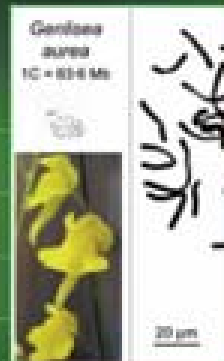


ANNALS OF BOTANY

Plant
Genome Sizes

EDITORIAL OFFICES IN UK, AUSTRALIA, CANADA, CHINA, JAPAN AND

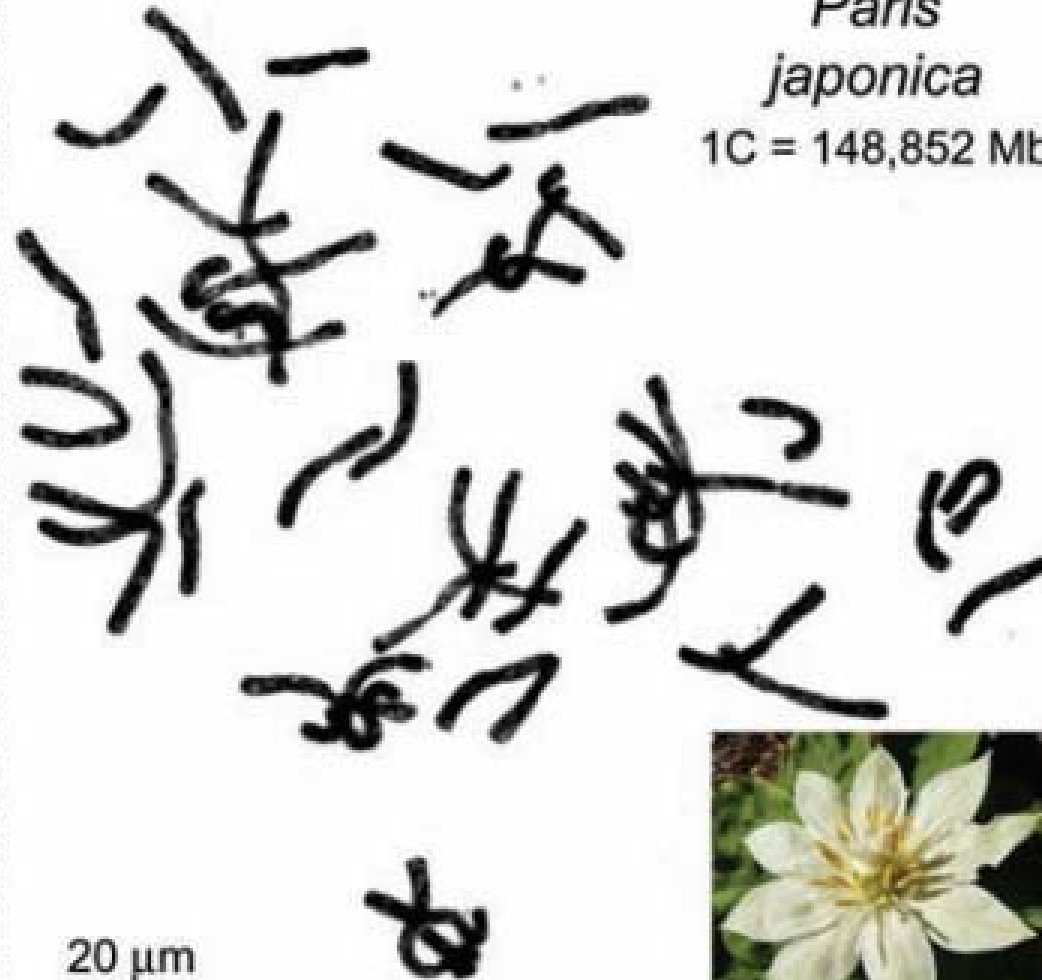
Genlisea aurea
1C = 63.6 Mb



20 μm

www.bot

Paris japonica
1C = 148,852 Mb



20 μm

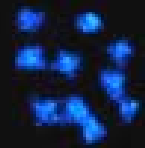


Volume 107

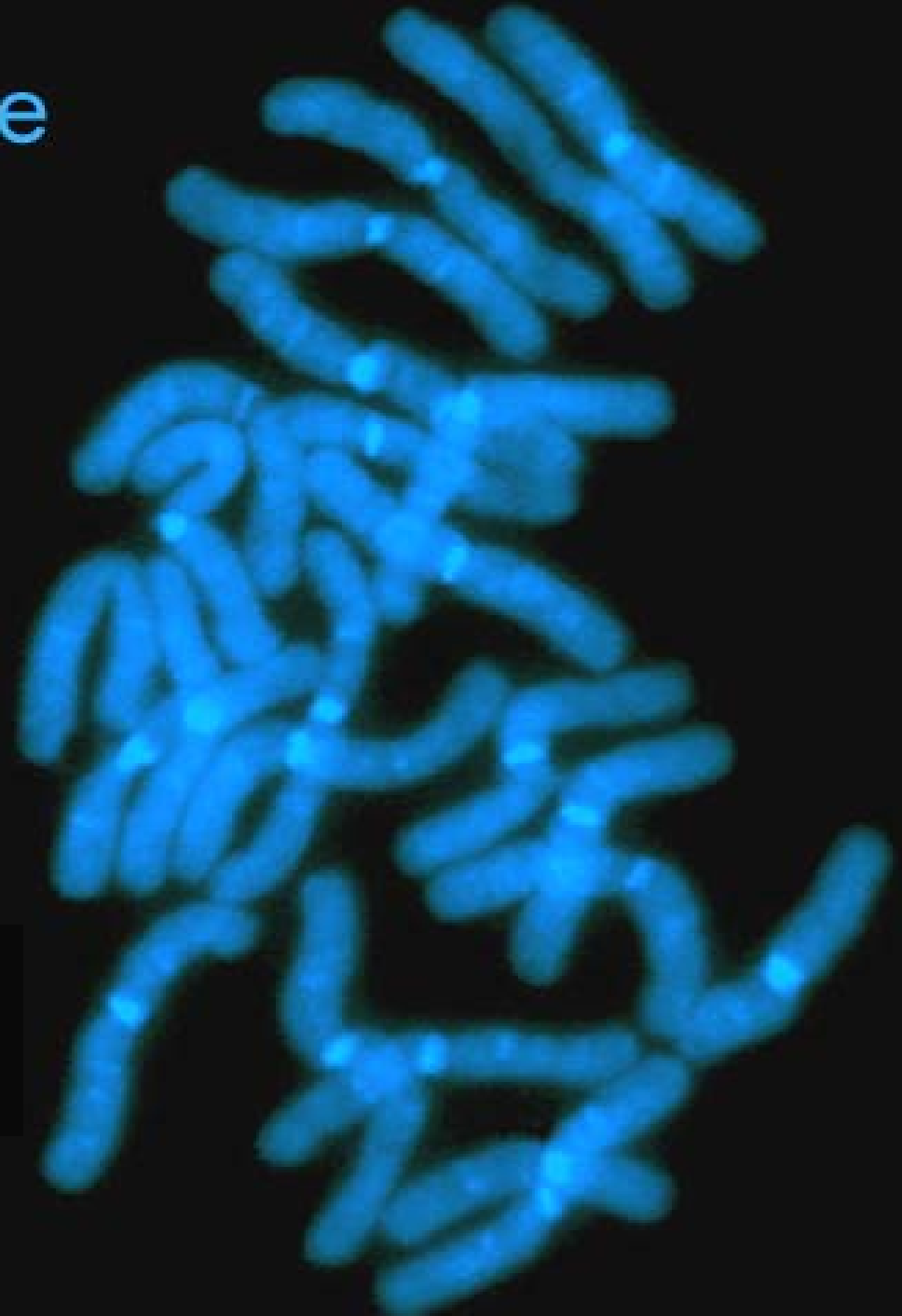
Number 3

March 2011

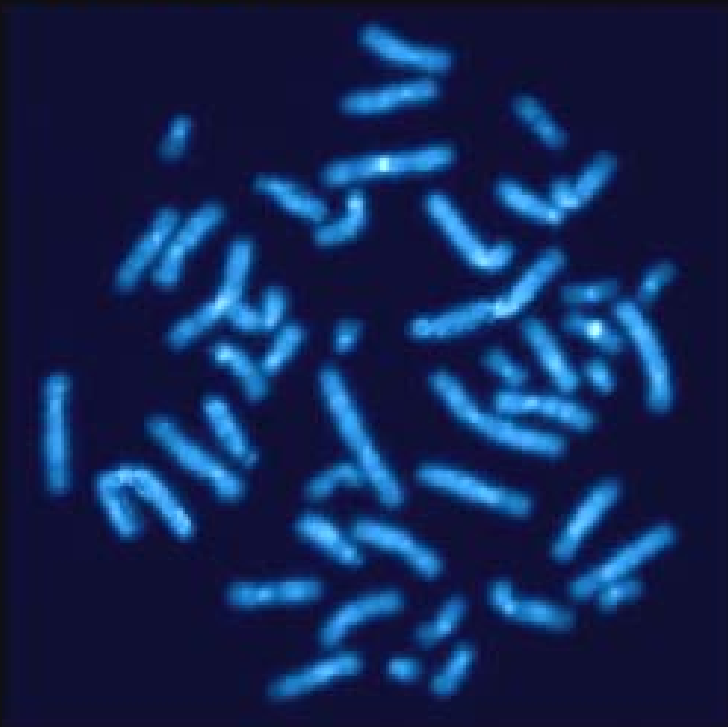
Arabidopsis



Pine



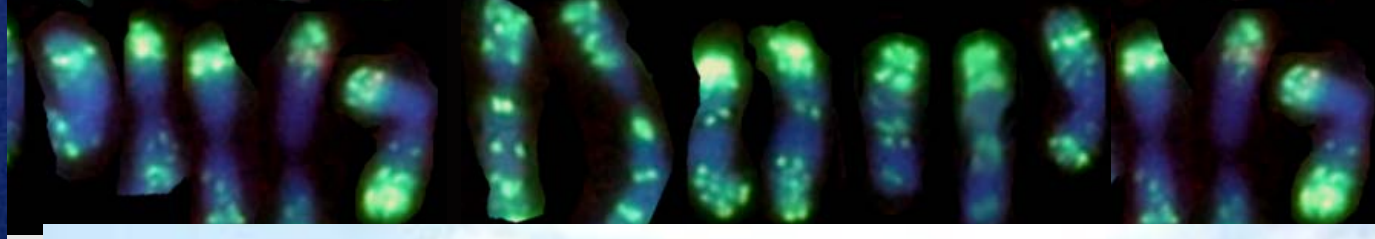
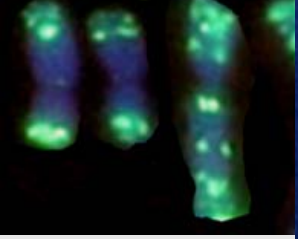
Human





Genome sizes: reading them out base-by-base

☛ HIV type 1 Virus	2hr 40 min
☛ Bacteria (<i>E. coli</i>)	53 days
☛ Yeast	138 days
☛ <i>Genlisea</i>	2 years
☛ <i>Arabidopsis</i>	5 years
☛ Man	100 years
☛ Wheat	5 centuries
☛ <i>Paris</i>	4 millennia





A special report on feeding the world

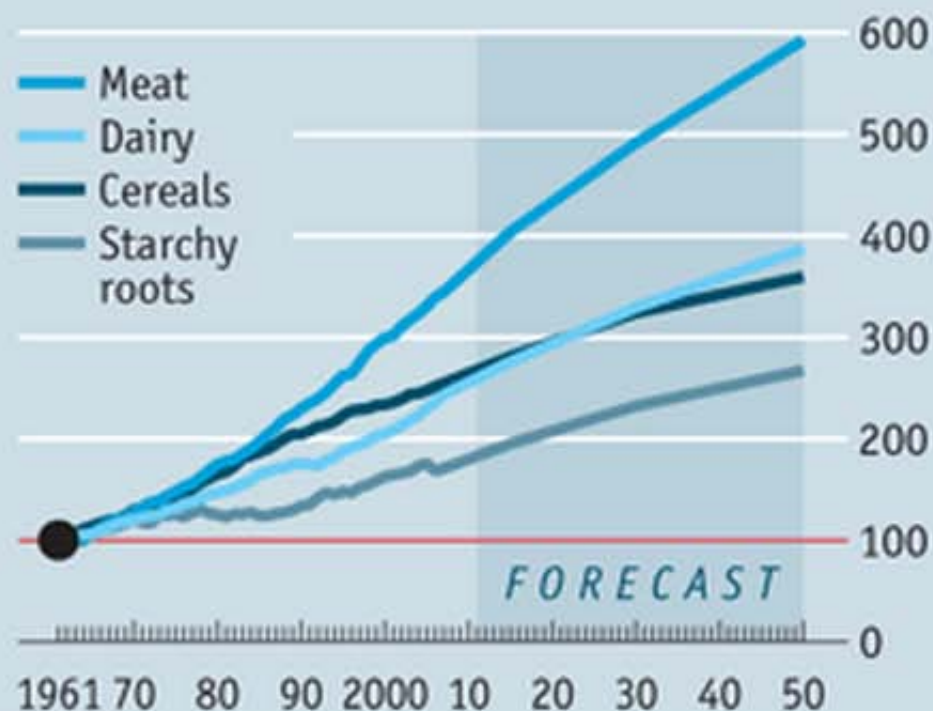
The 9 billion-people question

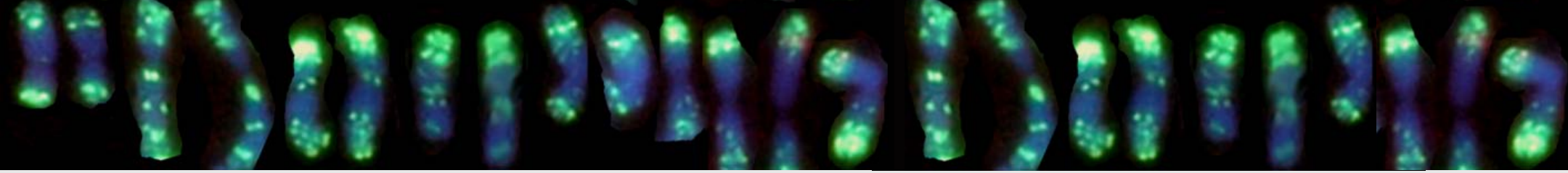
The world's population will grow from almost 7 billion now to over 9 billion in 2050. John Parker asks if there will be enough food to go round

Feb 24th 2011 | from the print edition



Global food demand, 1961=100





Molecular Cytogenetics

Part I:

- Chromosomes
- Genomes and their sizes
- DNA components of a chromosome
- Retroelements
- Chromosomes and sources of genetic markers

Part II:

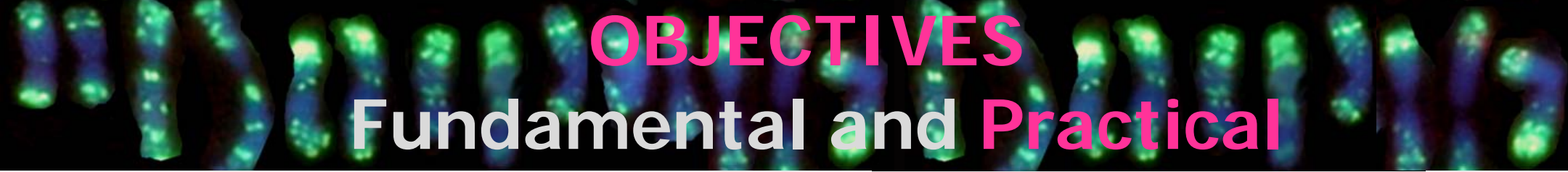
- Chromosome and genome painting
- Polyploids and cereal chromosome evolution
- Plant breeding and aliens



OBJECTIVES

Fundamental and Practical

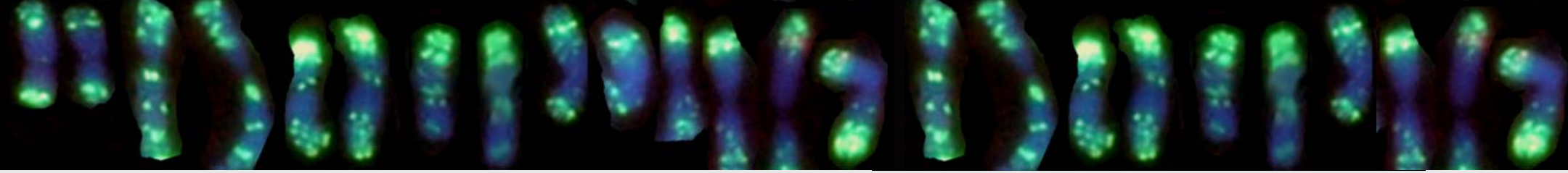
- ☞ Explain major structures and features of the DNA in genomes
- ☞ Understand the structure of chromosomes and genomes
- ☞ Explain the nature and origin of molecular markers
- ☞ Understand key events in evolution and generation of diversity including induced mutations



OBJECTIVES

Fundamental and Practical

- Understand how genomes and diversity can be manipulated and exploited
- Relate genome information and models to the applications in the genome of particular species
- Decide which molecular markers are appropriate for various applications
- Introduce the concept of superdomestication into breeding programmes and consider solutions to major problems facing breeders and farmers
- Use the literature relating to genomics, genetics and plant breeding and communicate it in writing



Molecular Cytogenetics

☞ Lecture

- 10.05-11.00
- 11.10-12.00

☞ Microscopy (Adrian 264 near computer room)

☞ Three slots: 3.00 - 3.30 - 4.00

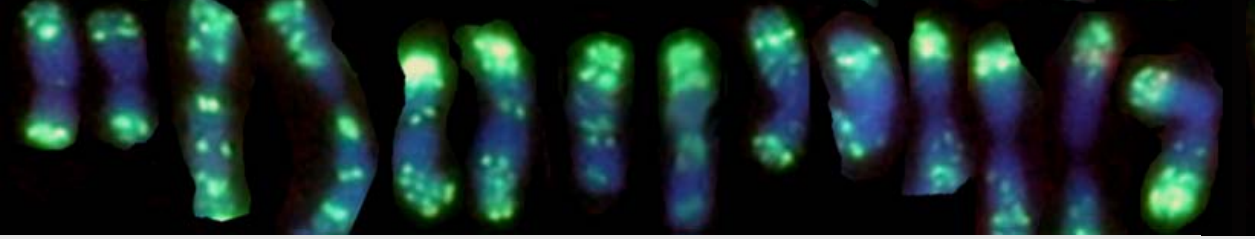


O₁

E₁ V₄

L₁ U₁ T₁

O₁ Z₁



**Nothing in Biology
Makes Sense
Except in the
Light of Evolution**

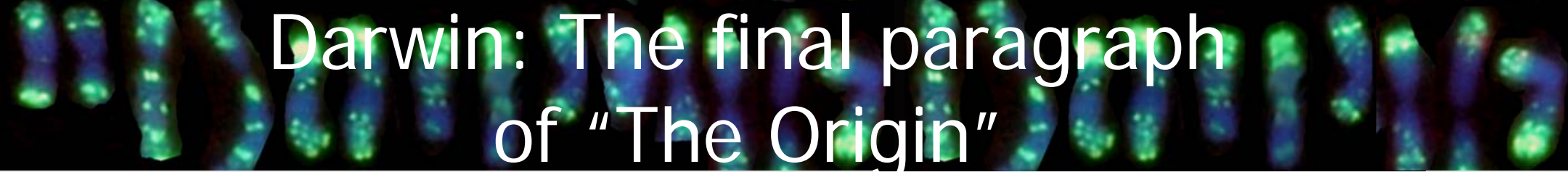
Theodosius Dobzhansky (1900-1975)

The American Biology Teacher, March 1973
(35:125-129).

Darwin: The final paragraph of "The Origin"

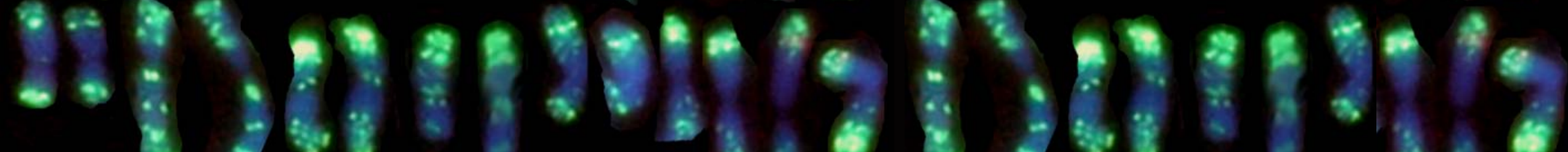
It is interesting to contemplate ... many plants of many kinds ... and to reflect that these elaborately constructed forms, so different from each other ...





Darwin: The final paragraph of "The Origin"

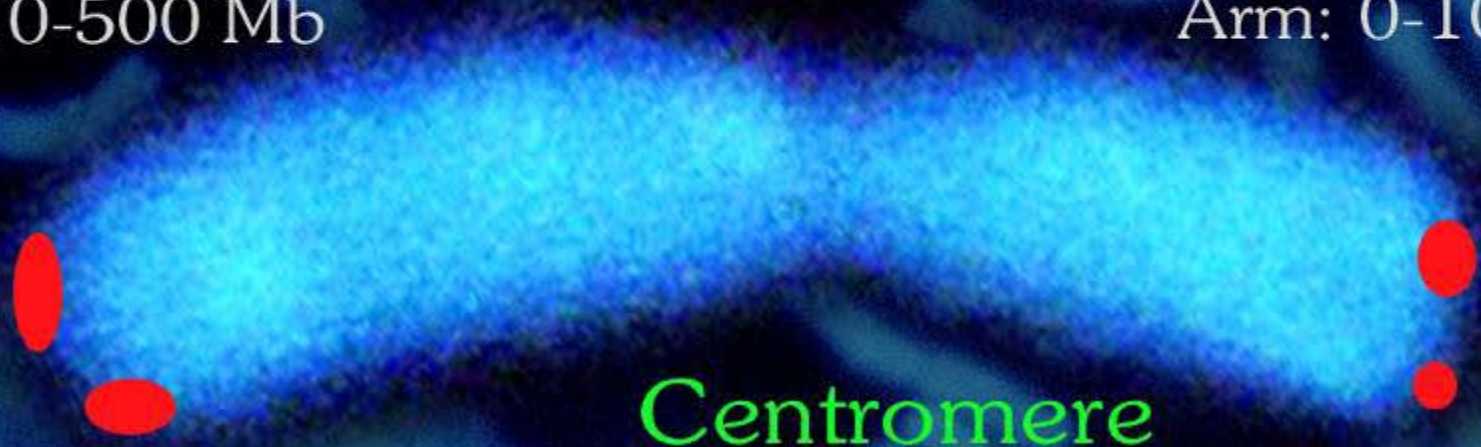
- It is interesting to contemplate ... many plants of many kinds ... and to reflect that these elaborately constructed forms, so different from each other ... have all been produced by laws acting around us ... from so simple a beginning endless forms most beautiful and most wonderful have been, and are being evolved.



Components of a Chromosome

Arm: 10-500 Mb

Arm: 0-1000 Mb



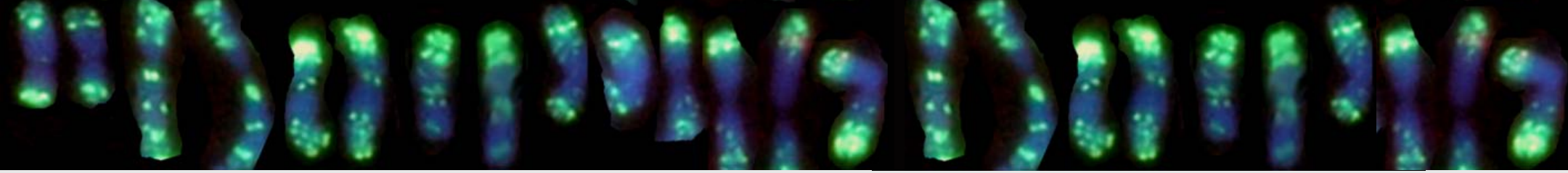
Centromere

Telomeres

Genes!

Packaging
- Division
- Organization

Replication and Transcription
- Enzyme access
- Regulation

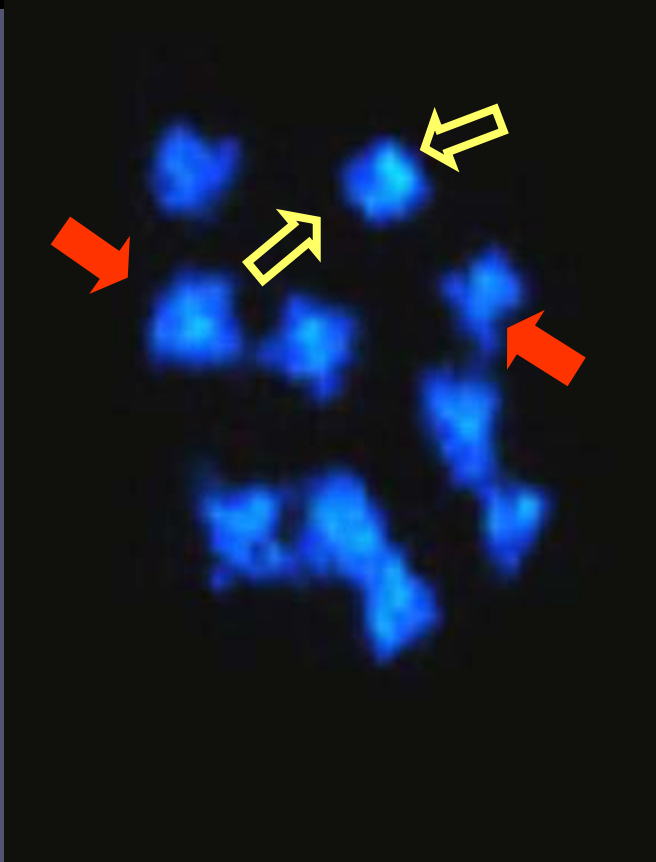


Chromosome



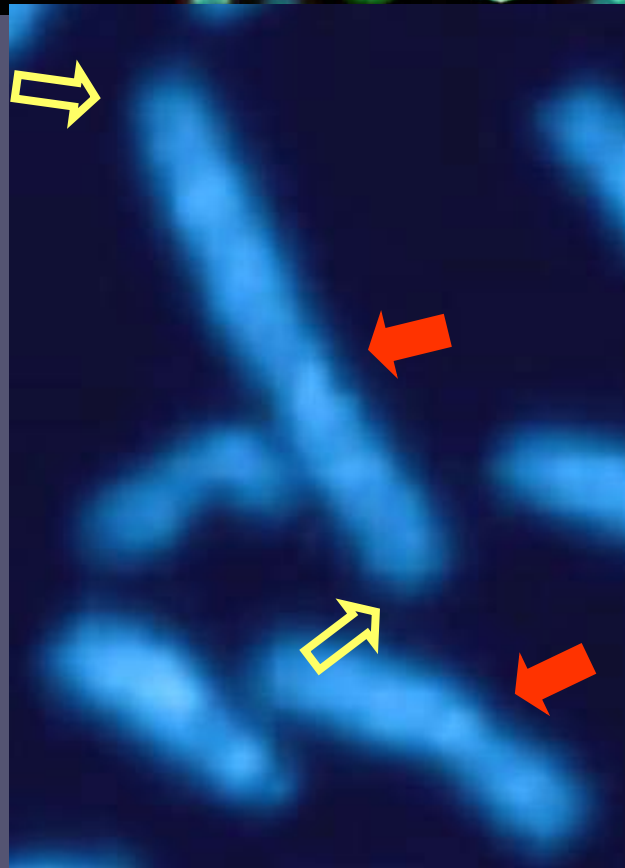
- ☞ Basic 'unit' of DNA in a cell
- ☞ Basic unit of heredity – Thomas Hunt Morgan 1911
- ☞ Macromolecule of DNA
 - very long, continuous
 - Contains genes, regulatory elements, other sequences
 - Structural components
- ☞ A broader definition of "chromosome"
 - includes the DNA-bound proteins which serve to package and manage the DNA.
- ☞ The word *chromosome* comes from the Greek *χρώμα* (*chroma*, colour) and *σώμα* (*soma*, body) due to its capacity to be stained very strongly

Somatic metaphase chromosomes



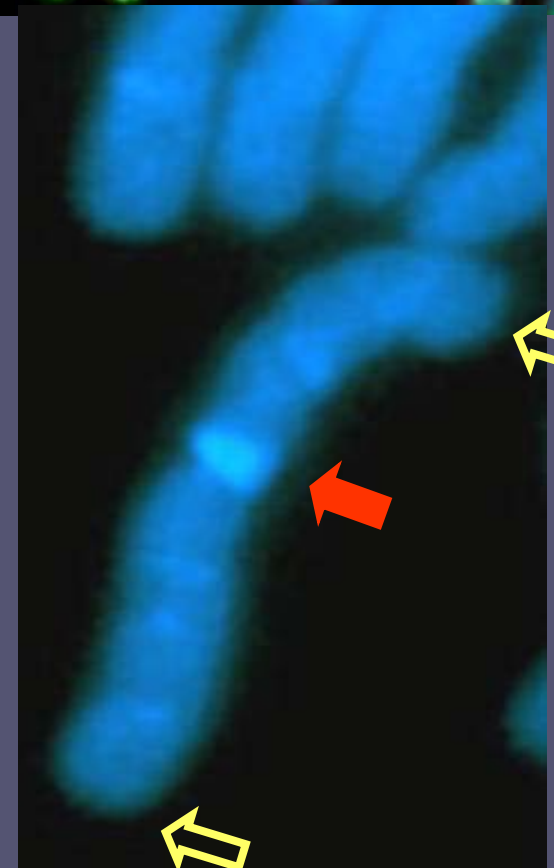
Arabidopsis

Centromere



Human

Telomere



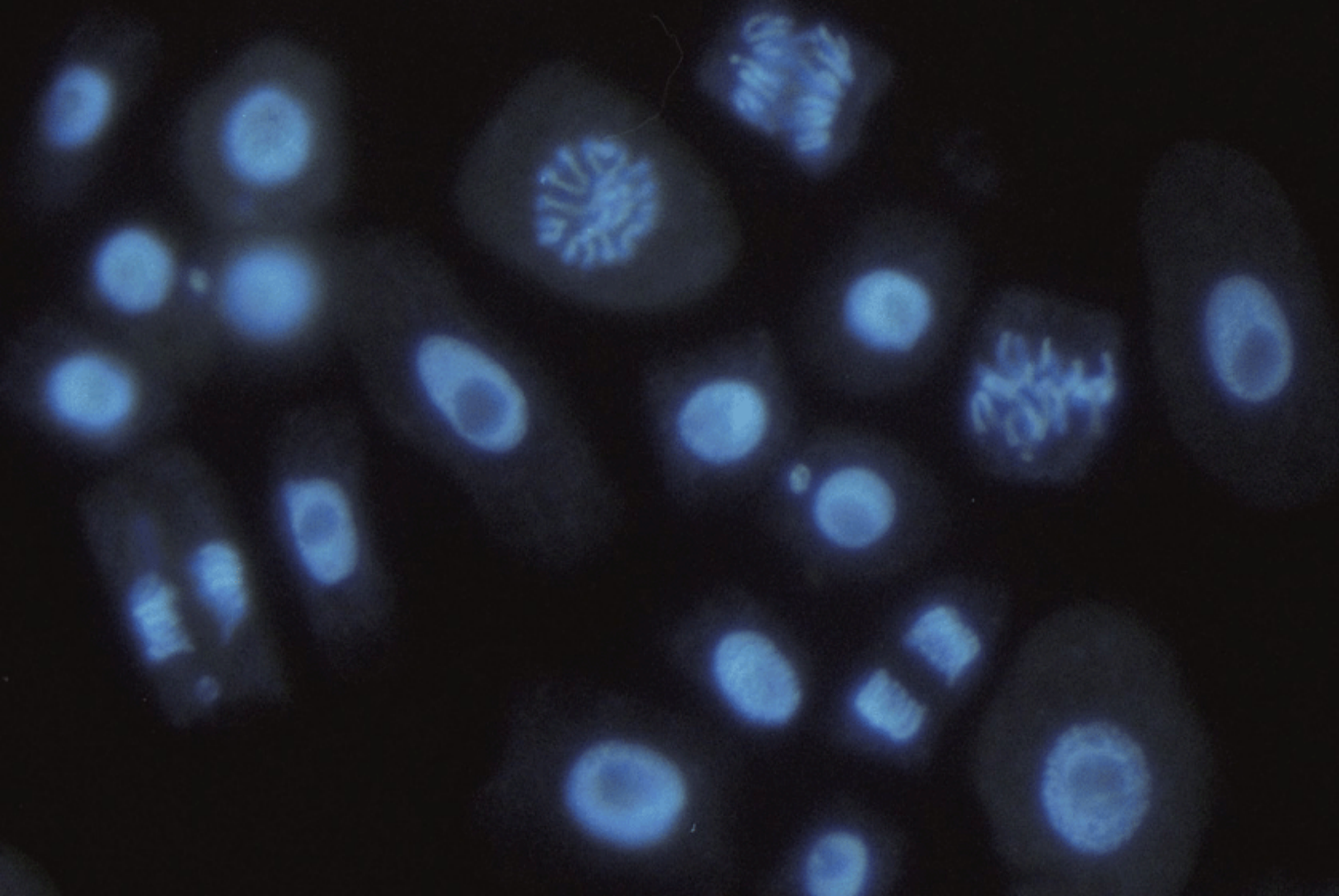
Pine



The Genome and Chromosomes

- ☞ The DNA is organized in multiple chromosomes
- ☞ Each is a single, linear DNA molecule
- ☞ The DNA is packaged around proteins (histones)
- ☞ The nuclear chromosome has special sequences at its ends

- ☞ There are separate genomes in the plastids and mitochondria
- ☞ There can be viral and bacteria-like genomes in the nucleus and cytoplasm



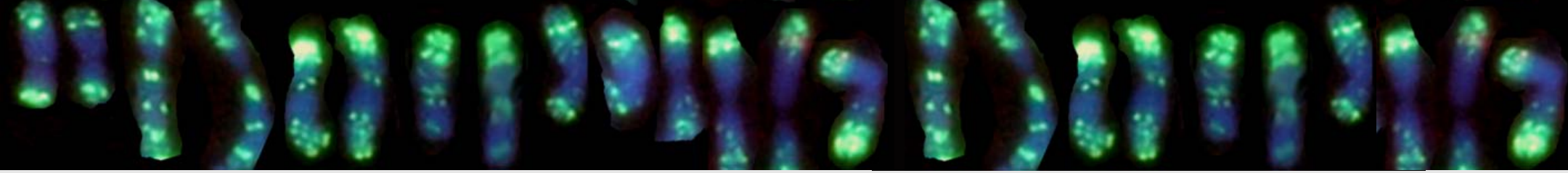


Repetitive DNA-Sequences form the largest part of the genome

<u>Species</u>	<u>Repetitive DNA</u>	<u>Genome size</u>
<i>Arabidopsis thaliana</i>	>25%	145 Mbp
<i>Sugar beet Beta vulgaris</i>	63%	758 Mbp
<i>Broad bean Vicia faba</i>	85%	12000 Mbp
<i>Rye Secale cereale</i>	92%	8800 Mbp
<i>Onion Allium cepa</i>	95%	15100 Mbp

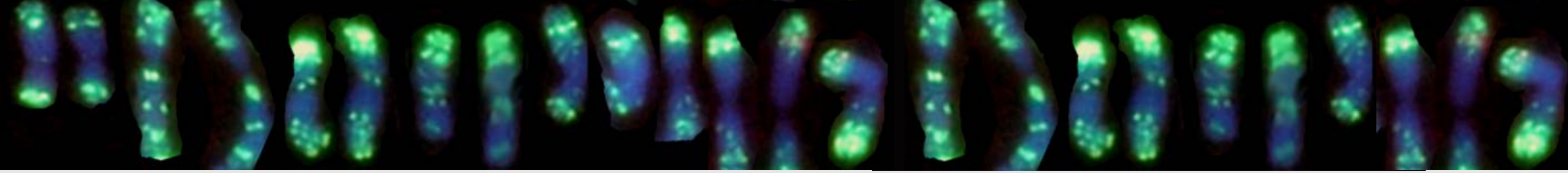
These species are all diploid – 2x

<i>Human Homo sapiens</i>	35%	3000 Mbp
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Genome

- ☛ Genes and regulatory sequences make up a small proportion of the genome
- ☛ The majority of DNA sequences in all higher eukaryotic genomes are repetitive sequences (50-90%)
- ☛ JUNK – no?; FUNCTION – largely unknown



What is the rest of the genome?

☞ Repetitive DNA

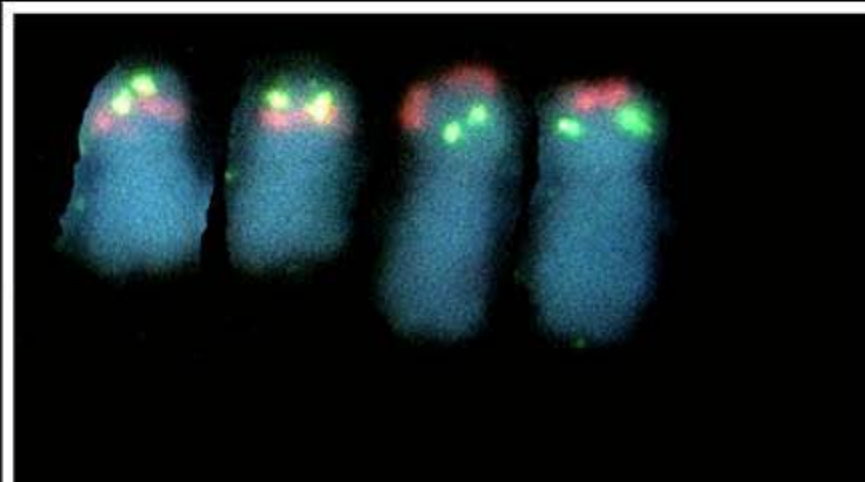
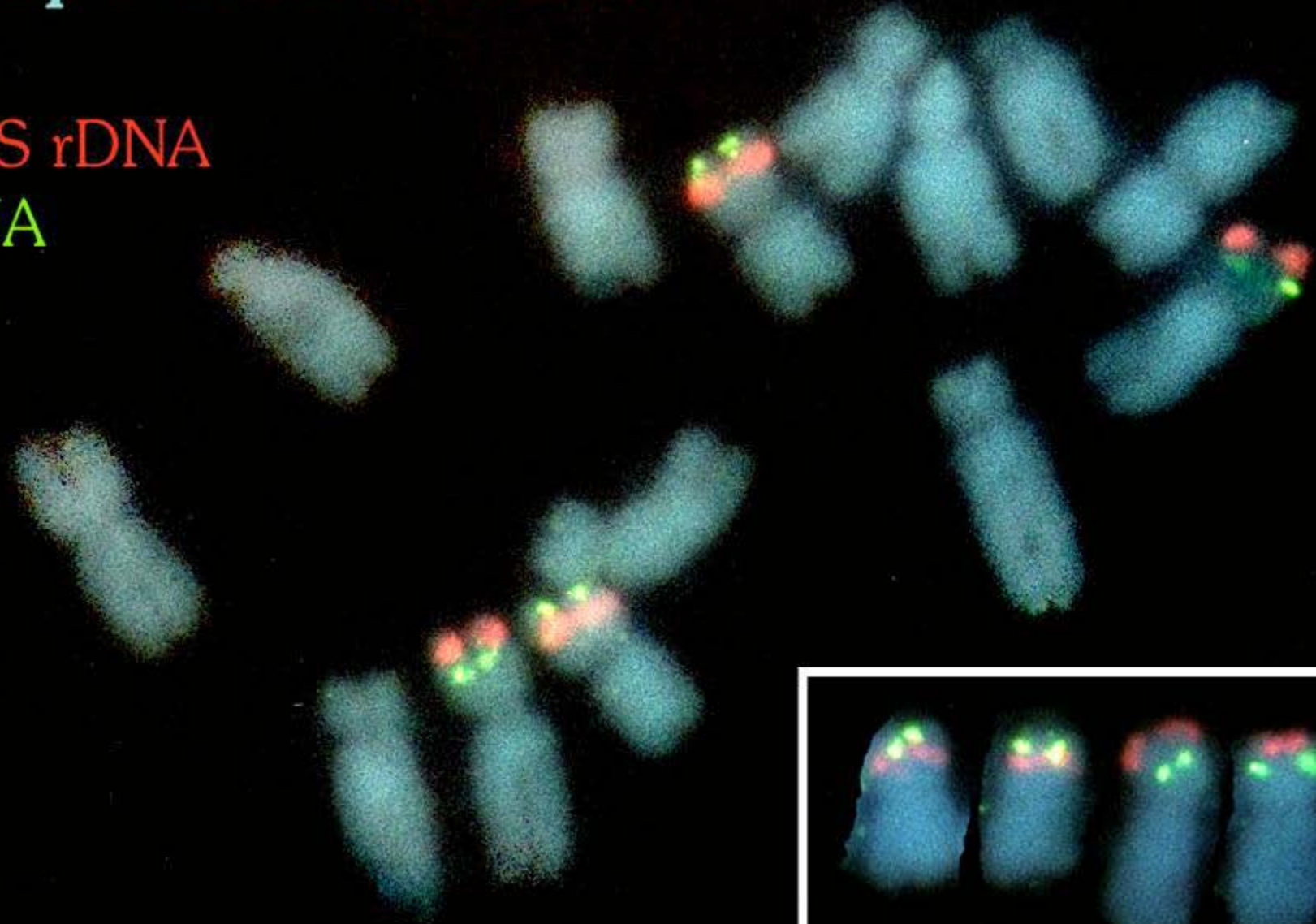
- Repeated genes
 - rDNA (45S and 5S)
- Sequence motifs from 2 to 20,000 bp long ...
 - Repeated thousands of times in the genome

Aegilops umbellulata

DAPI

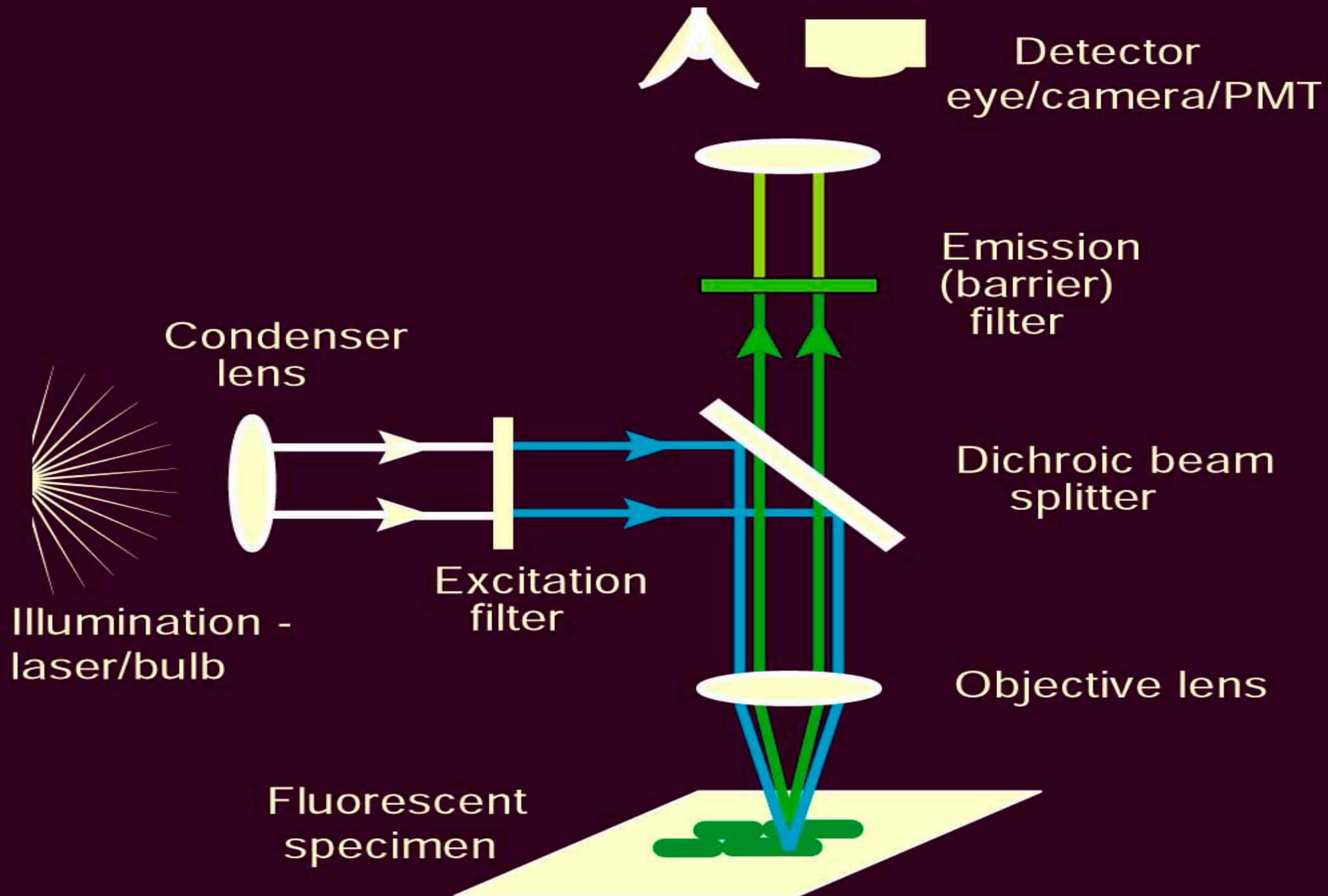
18S-25S rDNA

5S rDNA

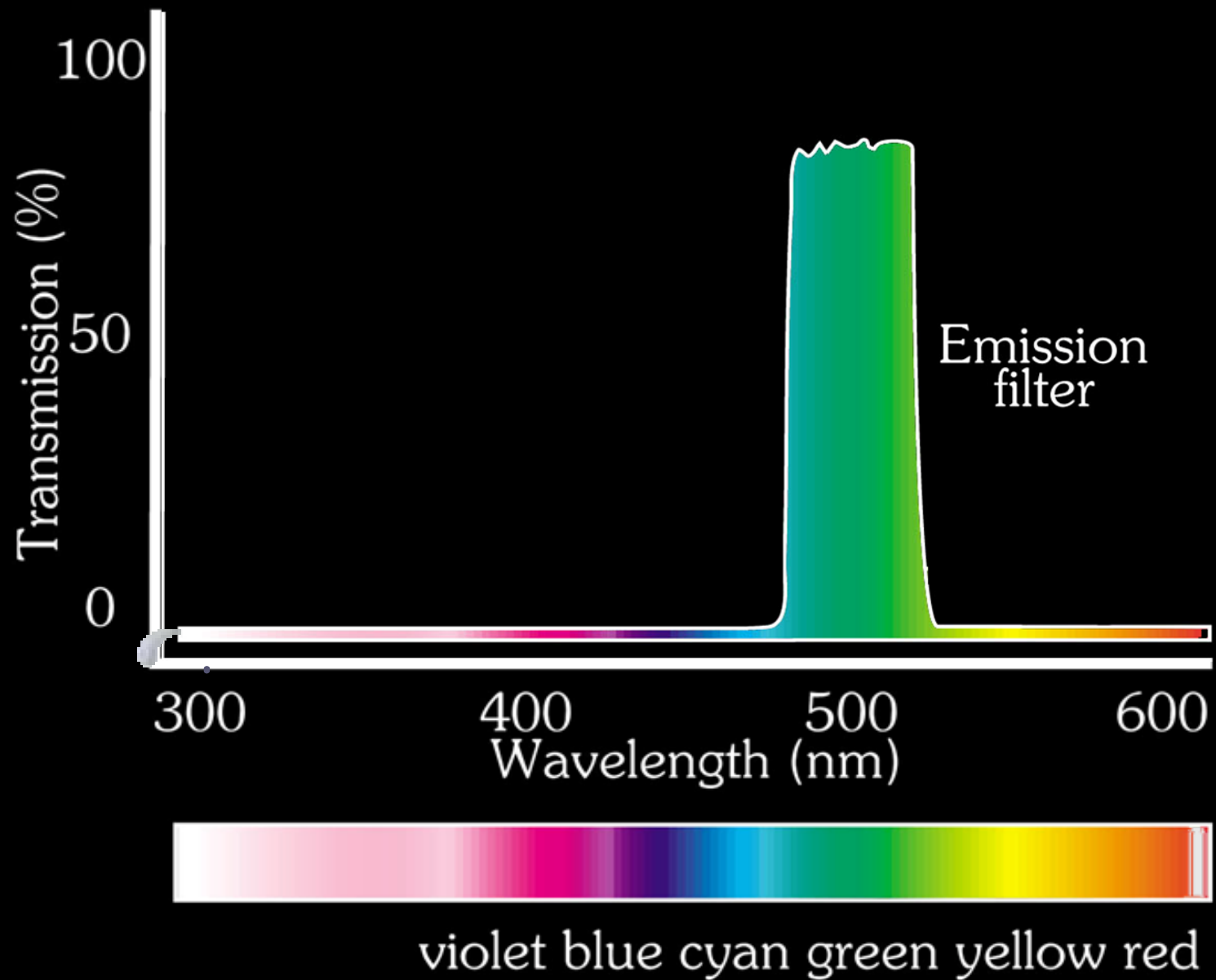


Fluorescence microscopy

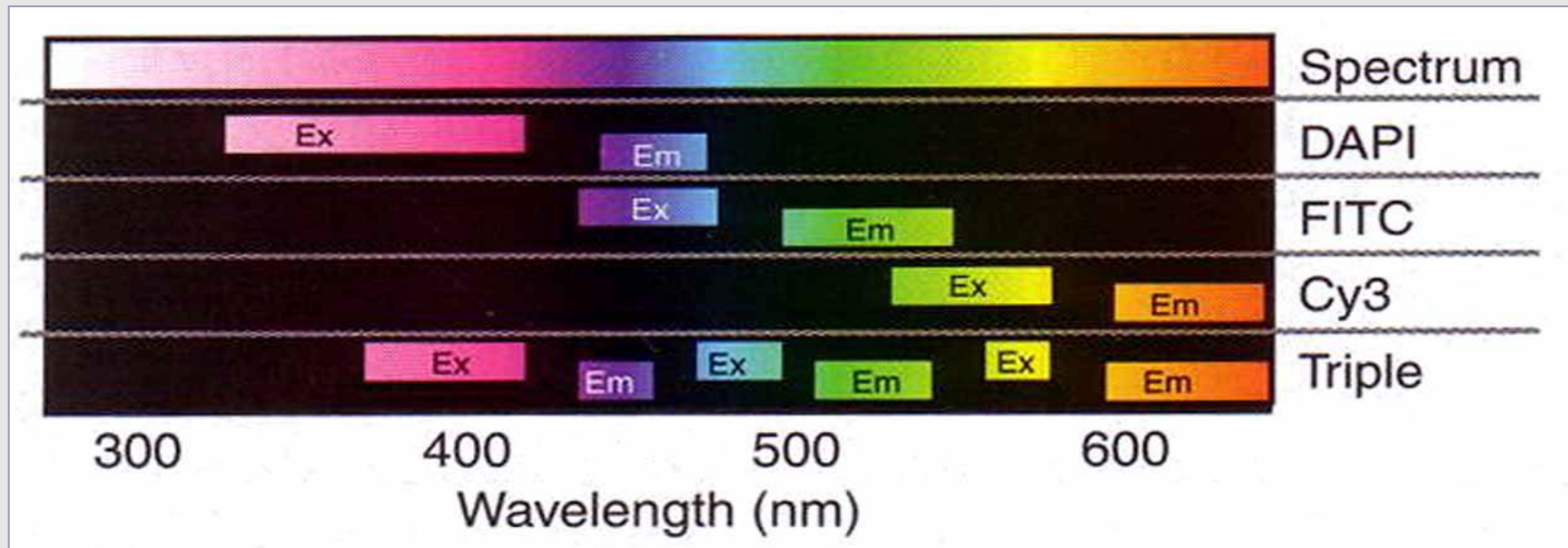




Fluorescence microscope filter properties



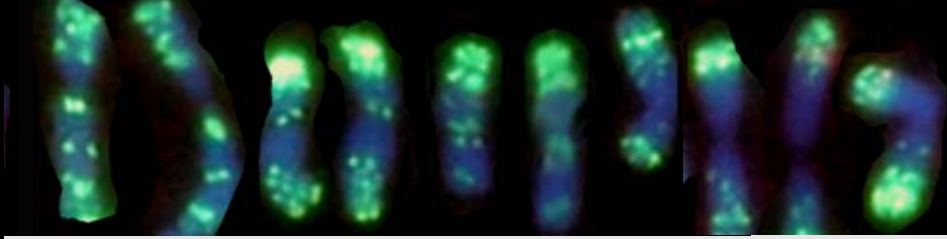
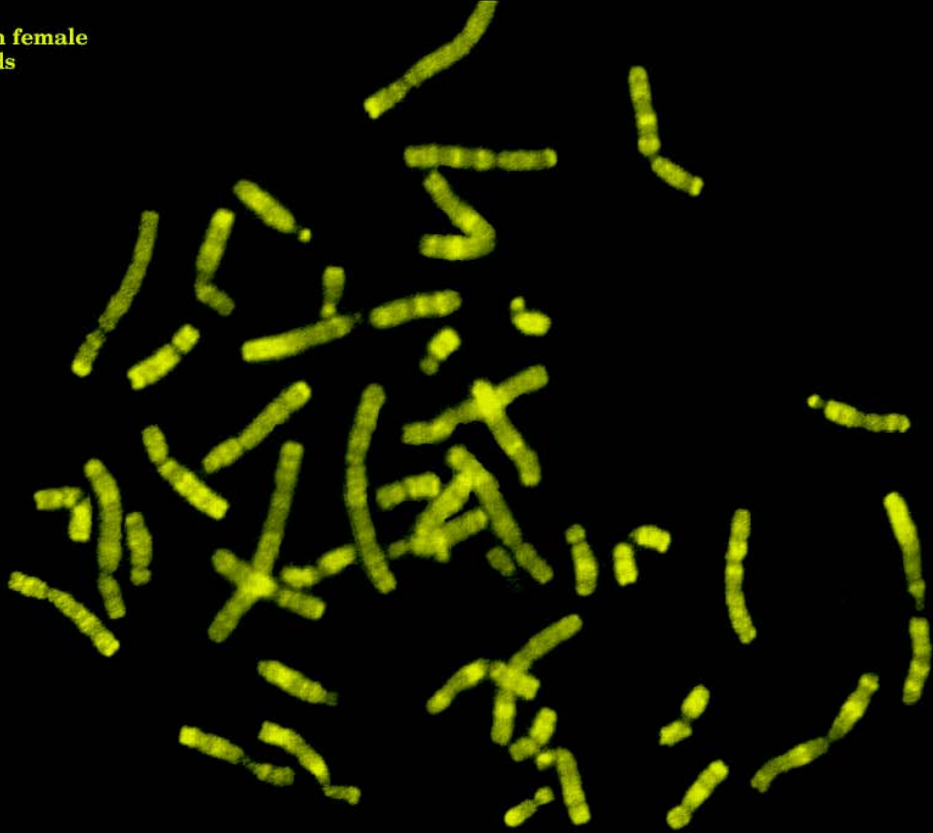
Fluorescence microscopy



Multi-colour FISH:

- Hybridizing two or more DNA probes simultaneously
- Multibandpass filters

Human female
R-bands

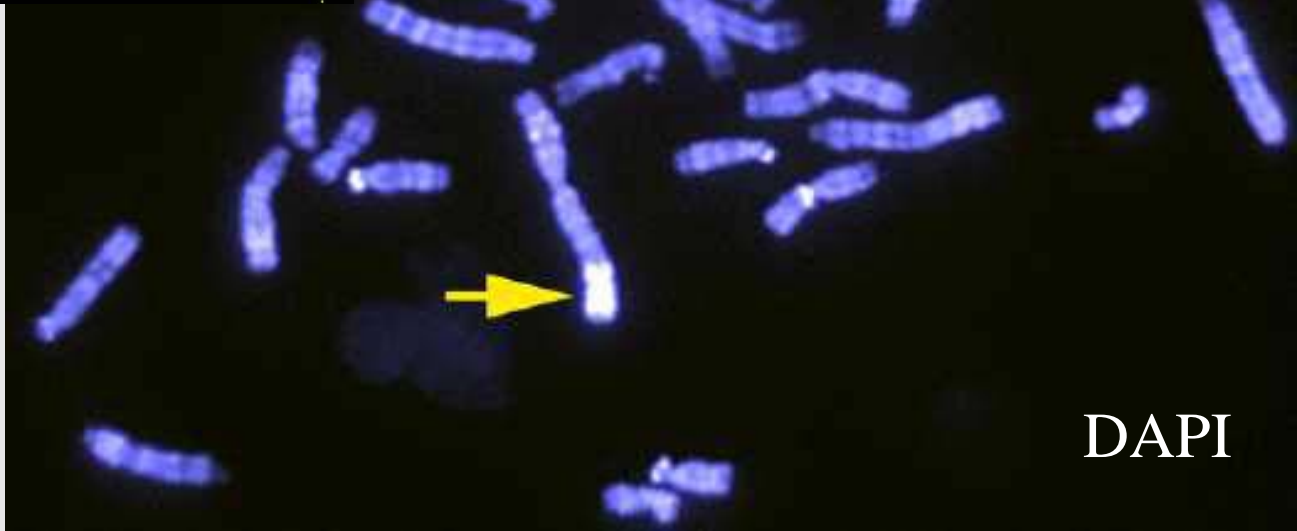
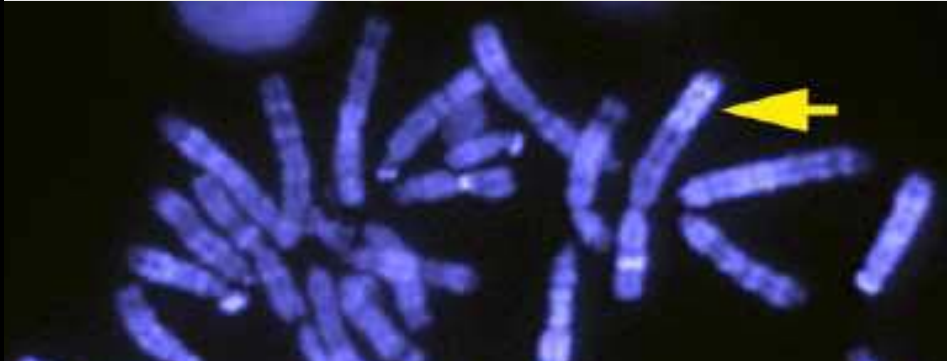


Fluorescent banding

DAPI: AT-rich

ChromomycinA3: GC-rich

Chromomycin A3: binds to GC rich DNA sequences

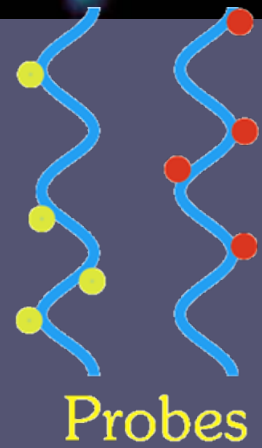


DAPI

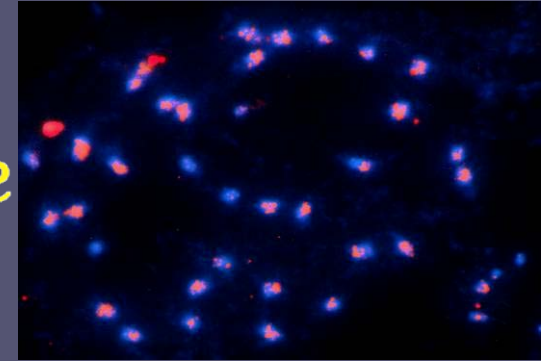
Triticale:
wheat x rye
hybrid



In situ (and array) hybridization technology



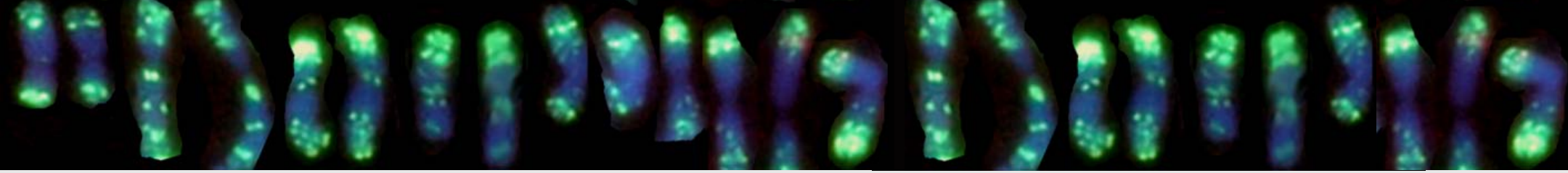
Hybridize



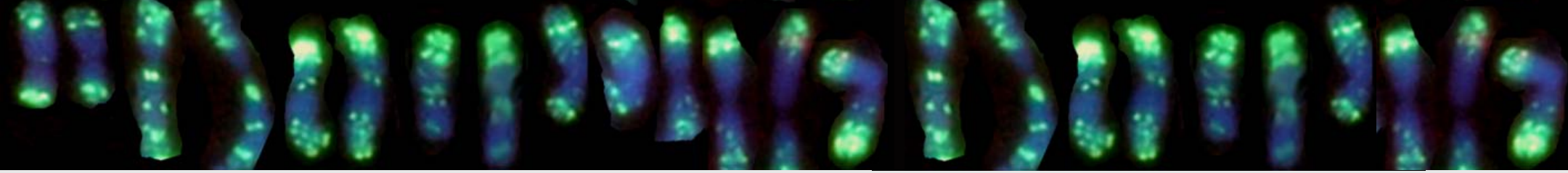
Detection of
Hybridization sites



Chromosome
preparation



What are the most important things you have learned so far?



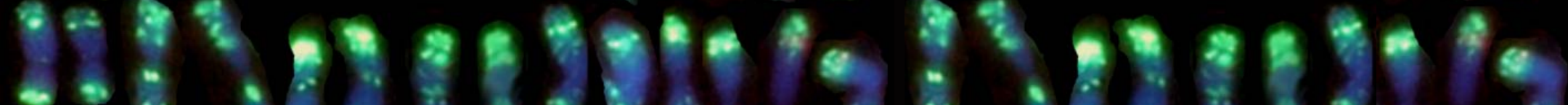
What are the most important things you have learned so far?

My answer: CONTACTS

... lecturers and other students.

SOURCES OF INFORMATION

... solving the overload



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[Language tools](#)

Google Search

I'm Feeling Lucky

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Pat Heslop-Harriso
available

David Ray

Options Add Contact

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- ★ [Comparing chromosomal and mitochondrial](#)
from [Chromosome Research \(Online First™\)](#)
- ★ [A Three-Dimensional RNA Motif in Potato](#)
from [The Plant Cell current issue](#)
- ★ [Improved reporting shows wider prevalence of](#)
from [Health Protection Agency](#)
- ★ [Pathh1: Plant Breeding: It's a Journey, Not a](#)
from [Twitter / Pathh1](#)

Science of the Invisible

- [Cracking the mysteries of R](#)
For the last two years I've been slowly working my way towards a new approach to teaching statistics. As my teaching load eases off I've finally...
- [Face to face #scieadr](#)
- [A discussion breaks out #oerbita](#)



BBC News - Science & Environ

- [UK rocket test for 1,000mph](#)
The first full test firing of the rocket that will power a British rocket to over 1,000mph (1,670km/h) will take place in the coming weeks.
- [EU 'won't change carbon target](#)
- [Nasa Glory launch ends in](#)

Nature - Issue - nature.com science feeds

- [The generation game](#)
- [Invest to diversify](#)

Weather


Nimes, Languedoc-Roussillon




14°C

Current: C
Wind: SW
Humidit


Search: annbot

 Link: Feeding the World – who pays? <http://dlvr.it/JQG9d>

annbot, [+]
Sun 06 Mar 12:52 via dlvr.it

 Mechanical perturbation of Arabidopsis <http://dlvr.it/JFF1m>


annbot, [+]
Fri 04 Mar 13:27 via dlvr.it

 Darwin reviews reviewed <http://dlvr.it/J82zN>


annbot, [+]
Thu 03 Mar 13:04 via dlvr.it

 ANNbotRT


Search: pathh1

 Feeding the World - who pays? Alun Cann's commentary on The Economist article on the 9 billion people question. <http://t.co/VdB3e44>

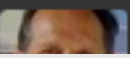
Pathh1, [+]
Sun 06 Mar 15:16 via FriendFeed

 Lots of good points on informal learning, social networks and teaching from Steve Wheeler, Plymouth. tks @rusource <http://slidesha.re/e4Go8H>


Pathh1, [+]
Sun 06 Mar 10:50 via web

 Plant Breeding: It's a Journey, Not a Destination. From US National Assn of Plant Breeders. <http://bit.ly/iaoR79>


Pathh1, [+]
Fri 04 Mar 15:42 via TweetDeck

 Read The Annbot Daily -

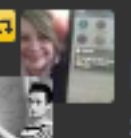
Search: chromosomes

 Photosynthesis, Homologous Chromosomes, Cellular Respiration, Oxidative Phosphorylation, Meiosis, Deoxyribonucleic Acid. #FML


• Anthony_WSUC, [+]
Mon 07 Mar 09:26 via txt

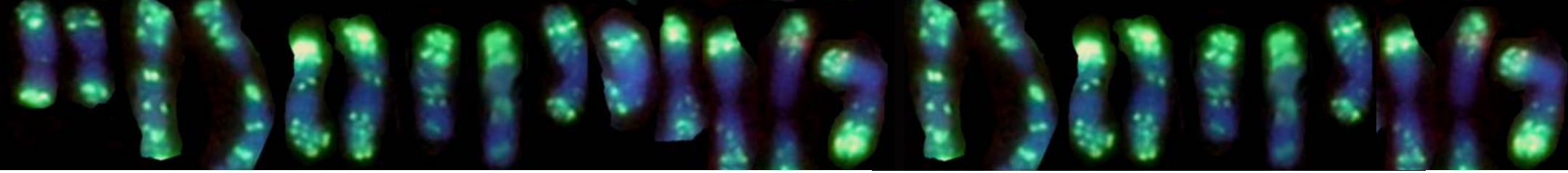
 Réplication des chromosomes et lutte contre le #cancer <http://goo.g/NS5W2> #biothérapies

• PierricChalois, [+]
Mon 07 Mar 09:11 via web

 Holy cow - read a great paper, was looking up more by same author - she uses Mendeley - just asked to connect. Wow. Just wow.

• kerryjcom, (RT by mendeley_com), [+]
Mon 07 Mar 11:49

 @Bronxbomber777 What comes

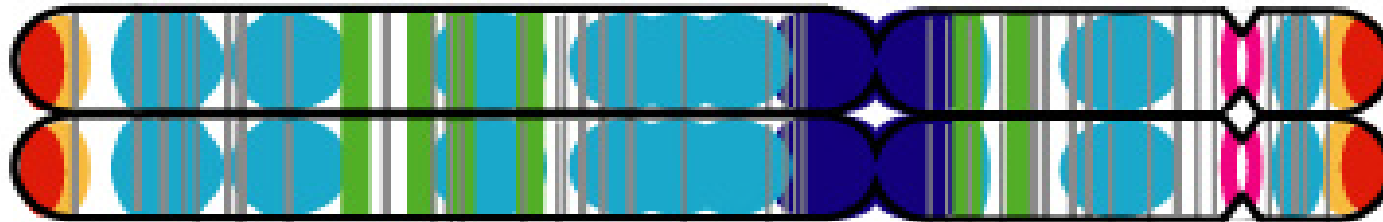


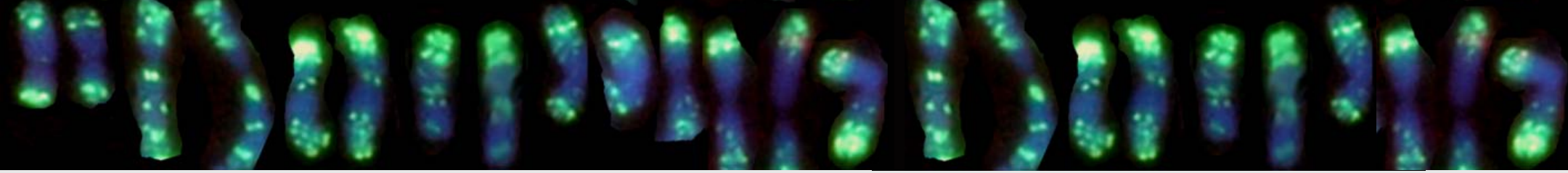
NOR: rDNA loci vary in number,
position and size



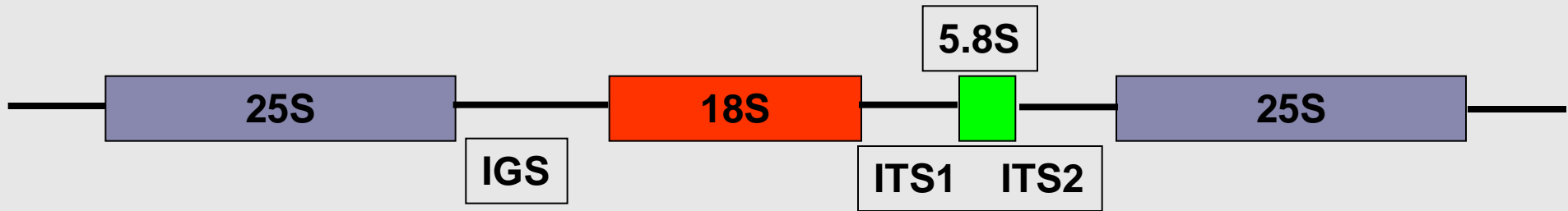
rDNA repeats

Chromosome
Satellite





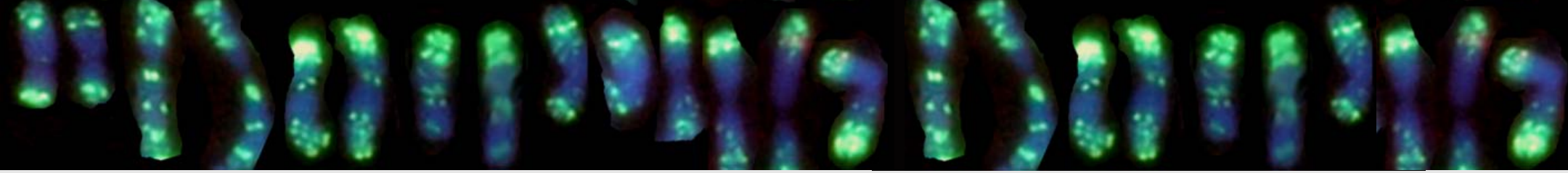
rDNA



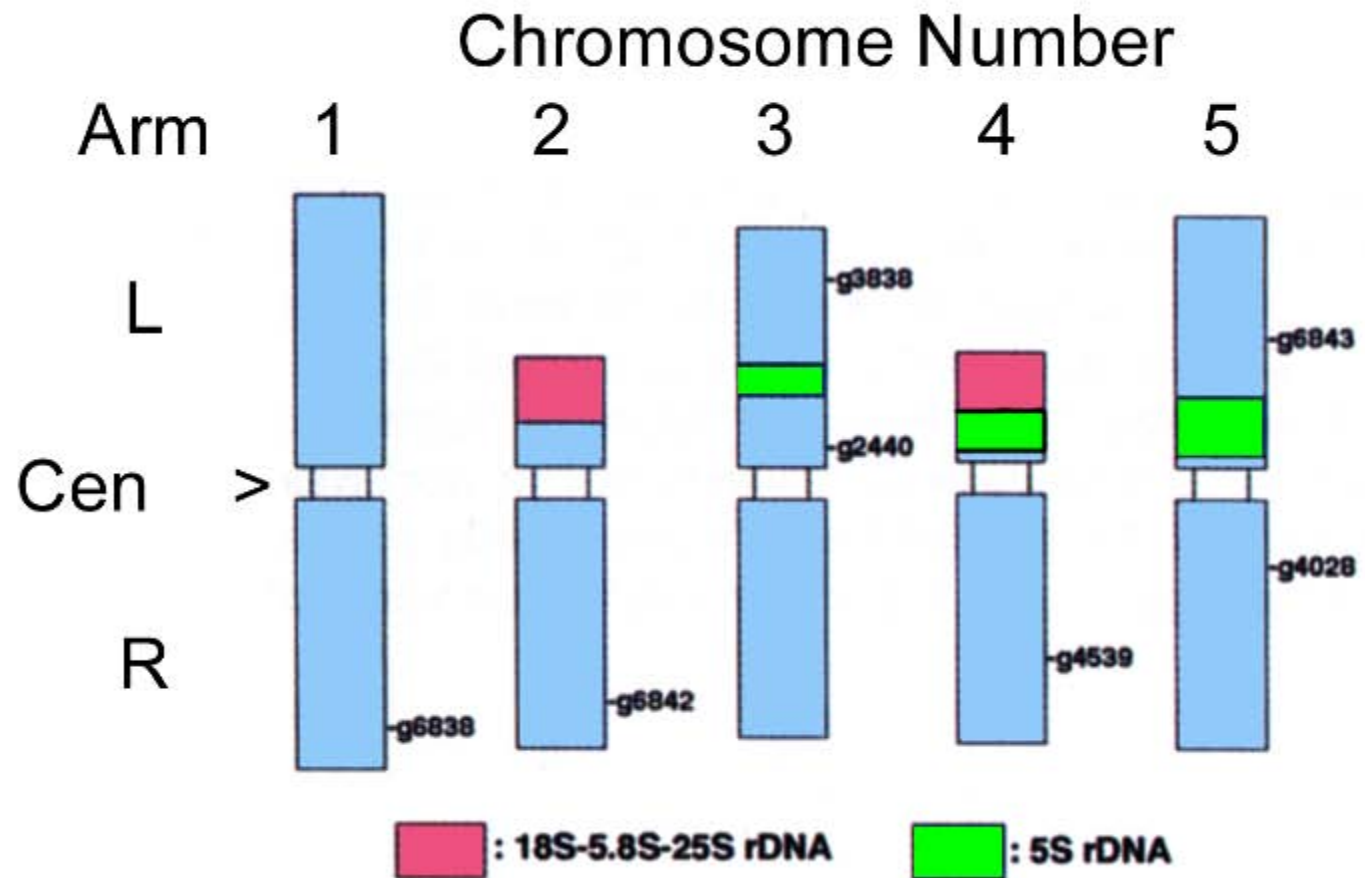
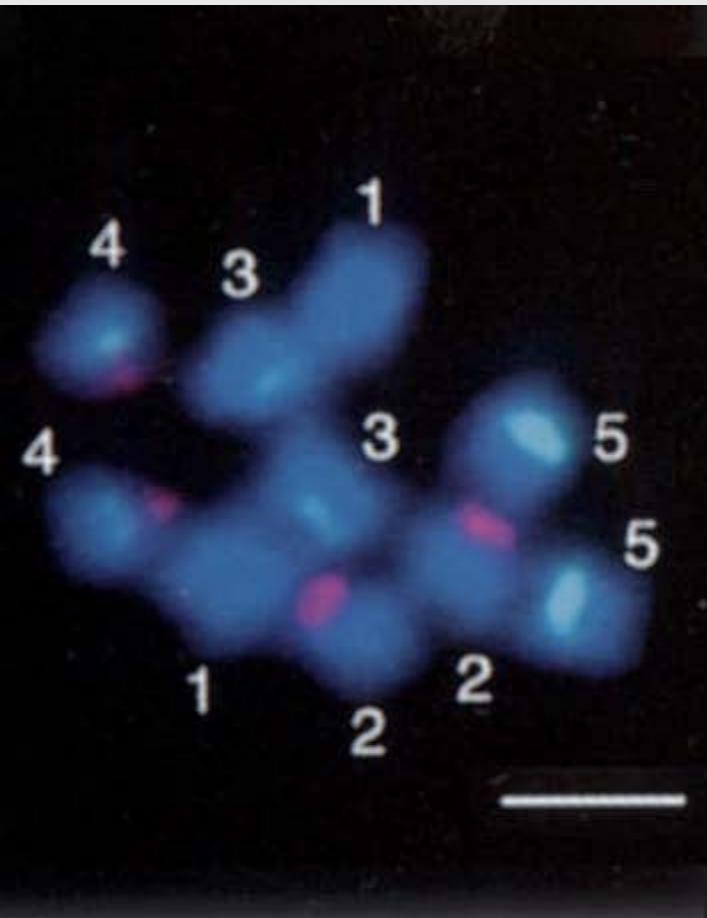
- ☞ Coding regions (nS) highly conserved, e.g. 18S gene of soybean shares 75% nucleotide homology with yeast.
 - Thus can compare over a long evolutionary distance
 - Can be used as anchors for PCR amplification
- ☞ Spacer regions highly variable
 - Species or below
 - Genus
 - Populations (sometimes)

IGS...InterGenic Spacer or
non-transcribed spacer

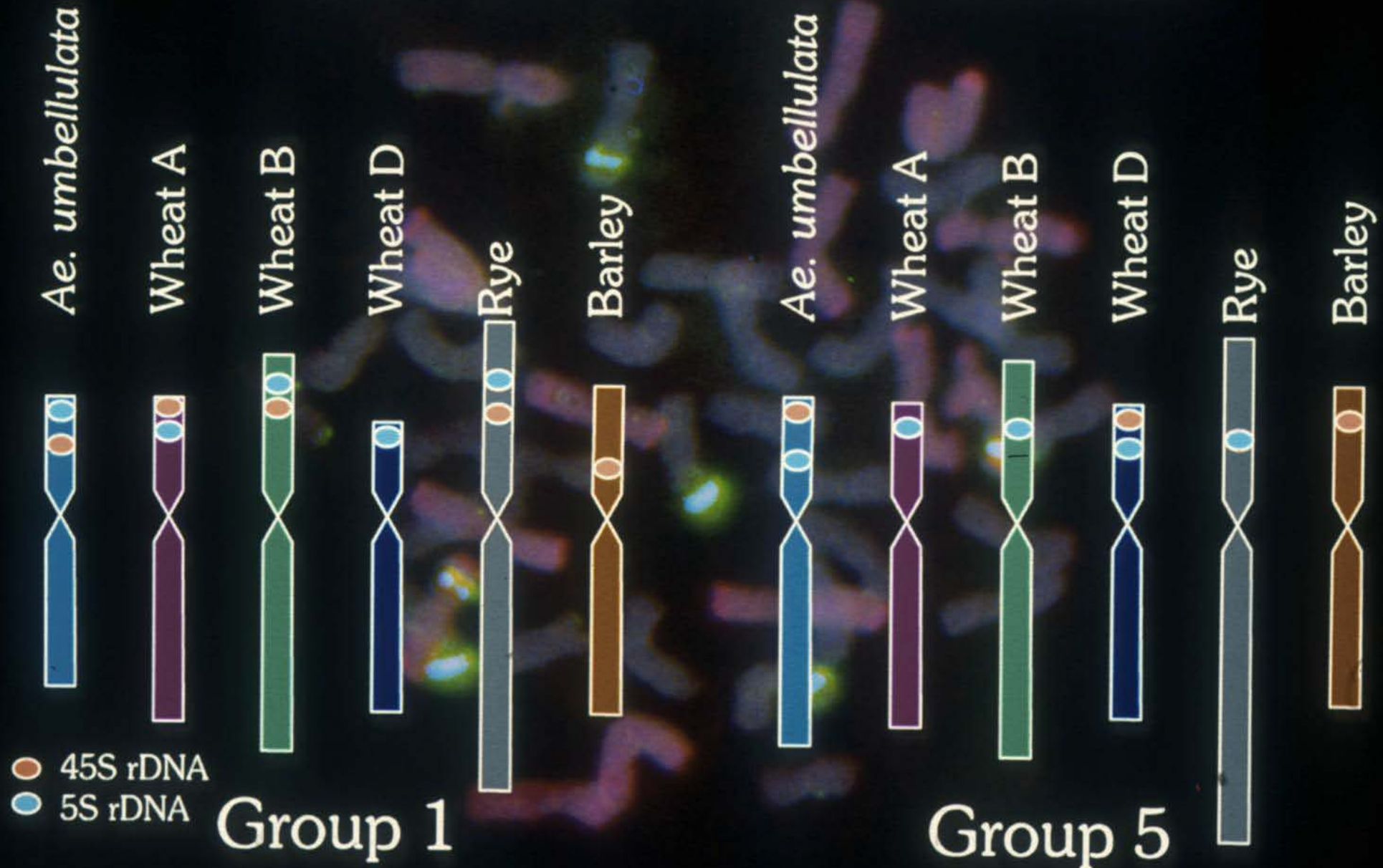
ITS...Internal Transcribed Spacer

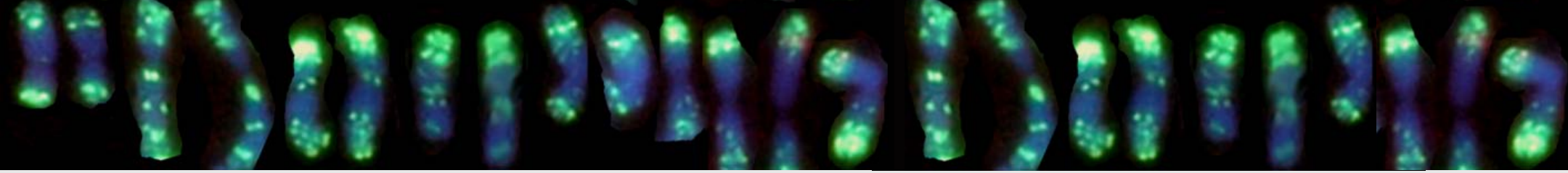


Arabidopsis thaliana $2n=10$



rDNA sites in Triticeae Genomes

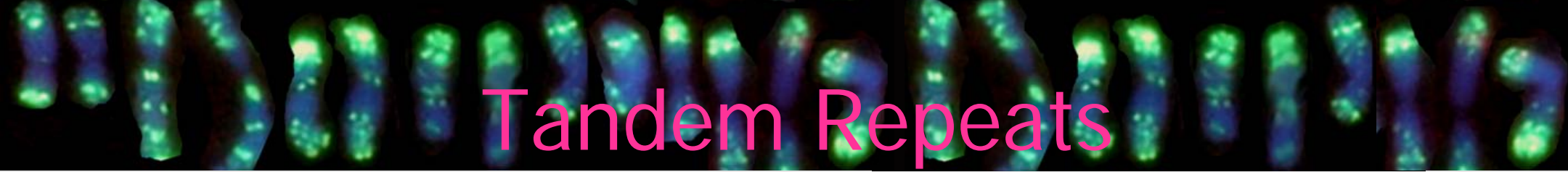




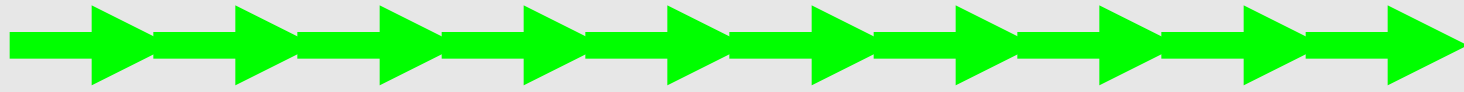
What is the rest of the genome?

☞ Repetitive DNA

- Repeated genes
 - rDNA (45S and 5S)
- Sequence motifs from 2 to 20,000 bp long ...
 - Repeated thousands of times in the genome



Tandem Repeats



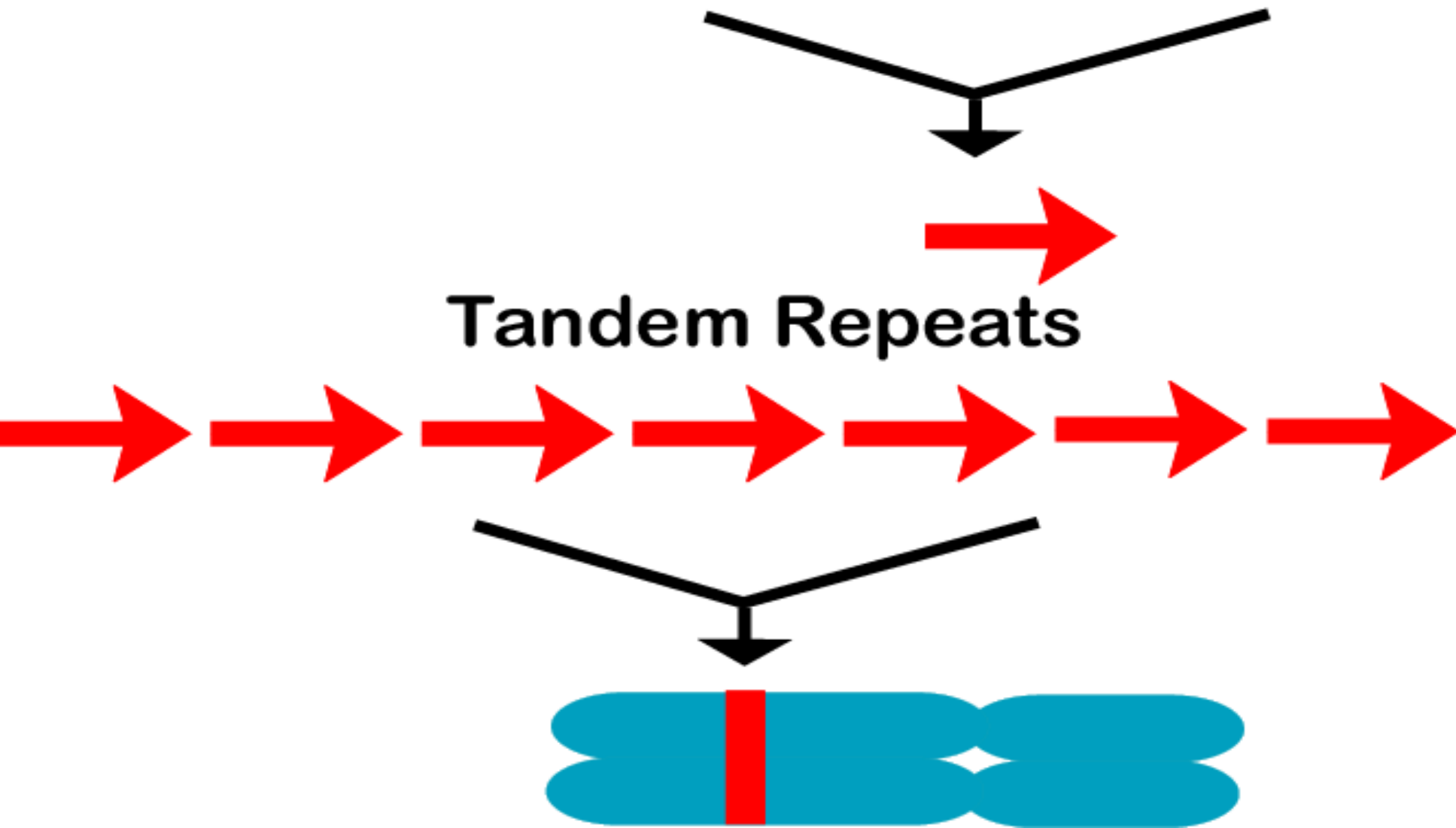
Where each arrow is a single unit of a repeat –
- often a multiple of 180 bp but up to 10kb long

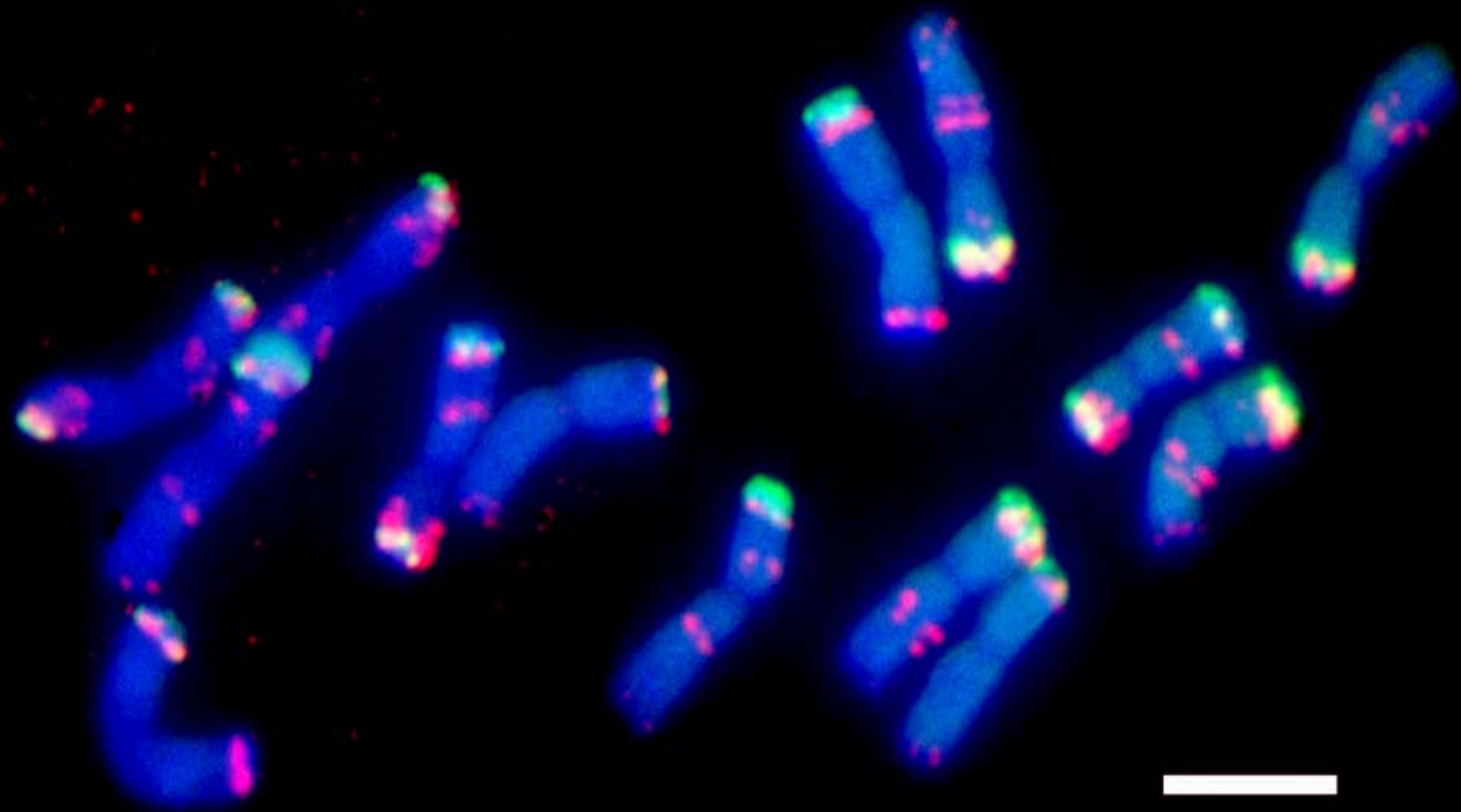
Head-to-tail organization

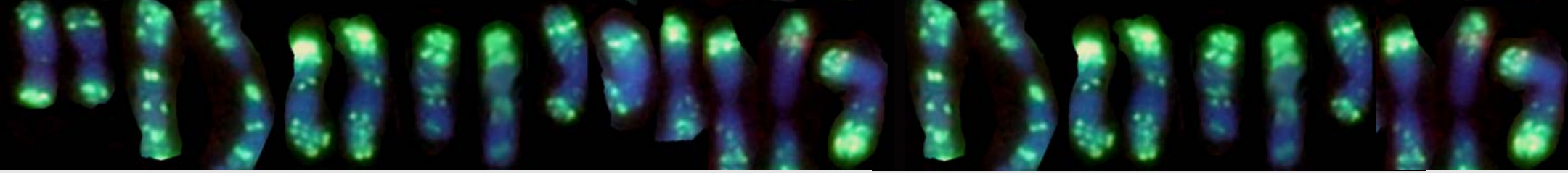
GCGCTAG GCGCTAG GCGCTAG GCGCTAG GCGCTAG GCGCTAG

Tandemly arranged DNA monomers

Repeat monomer: CCTAGCGTAACGGGTACGGGCTAGC







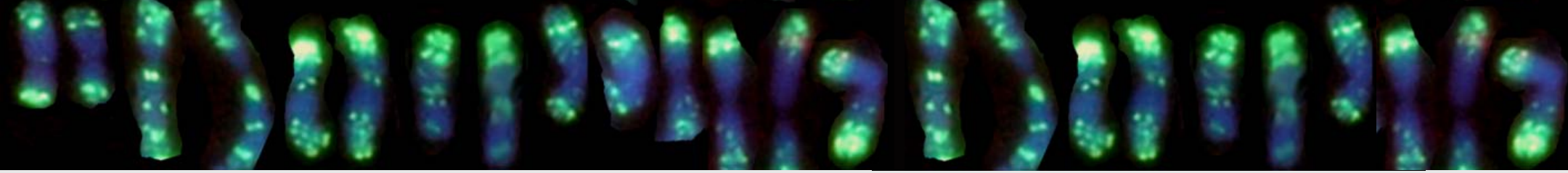
Repeated DNA sequences

motifs of 2-1000s of bp, repeated 10-10 000x

☞ Tandem repeats

- genes: rDNA, histones
- long tandem repeats or satellite sequences
- simple sequence repeats
 - Microsatellites
 - Minisatellites

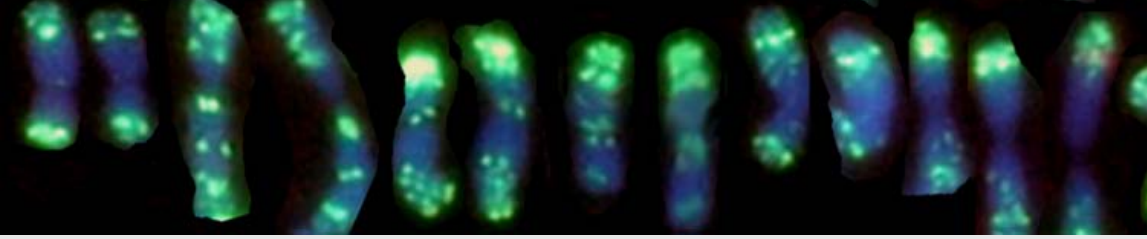
Satellite: shoulder on CsCl gradient centrifuge
or Band in restriction digest



Tandem Repeats

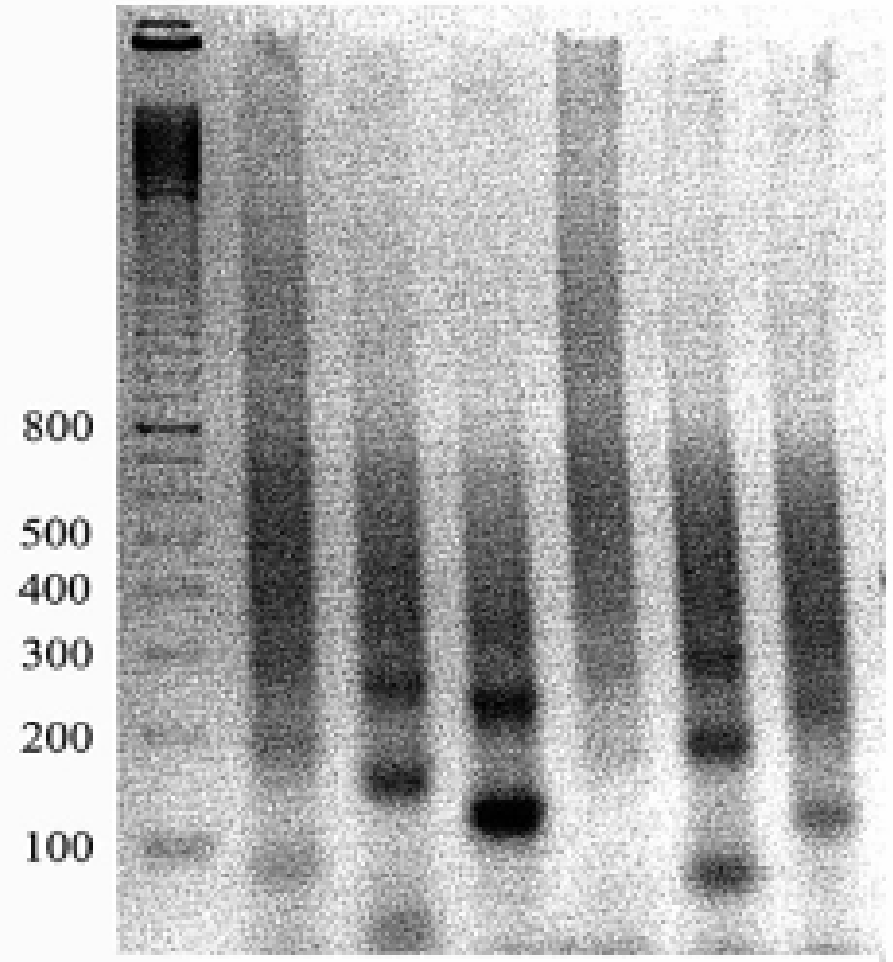


- Where each arrow is a single unit of a repeat – often a multiple of 180 bp but up to 10kb long
- GAGGCGTC GAGGCGTC GAGGCGTC GAGGCGTC
GAGGCGT**G** GAGGCGTC GAGGCGTC GAGGCGTC
GAGGCGTC GAGGCGTC GAGGCGTC GAGGCGTC

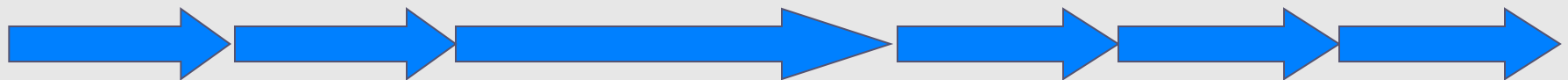


A

1 2 3 4 5 6



A complex sequence organization



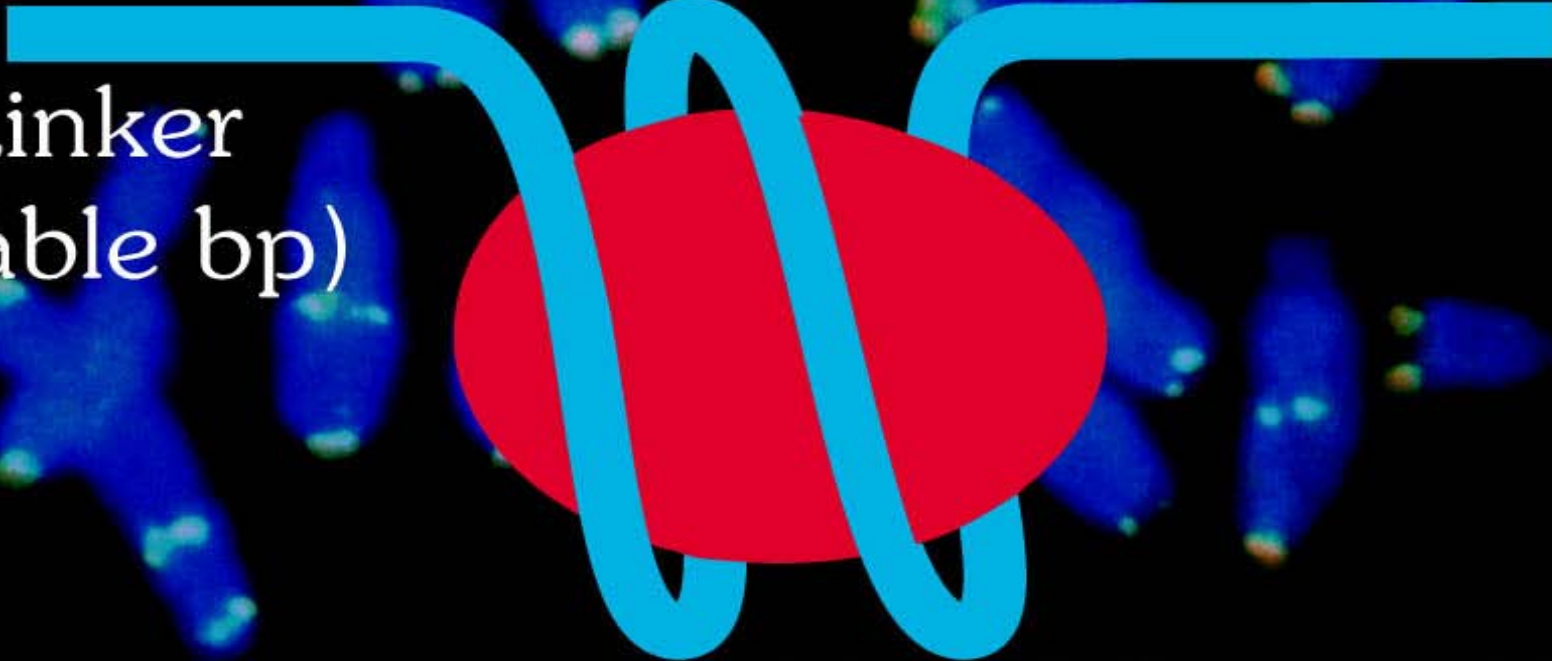
DNA packs around nucleosomes



Histone octamer



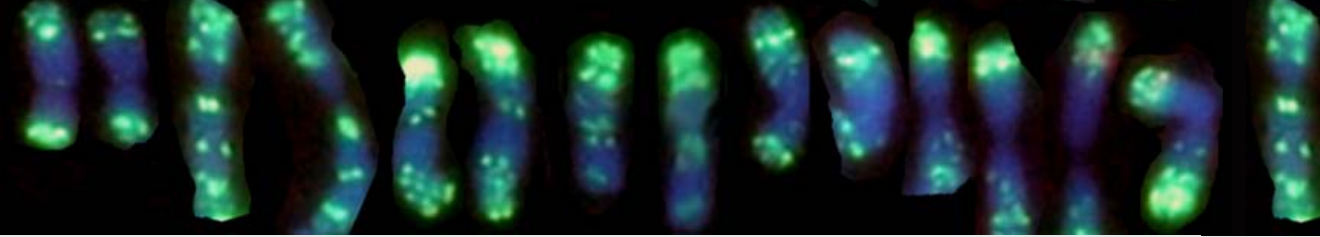
DNA double helix



Linker

(variable bp)

Two turns = 147 bp + linker to next nucleosome

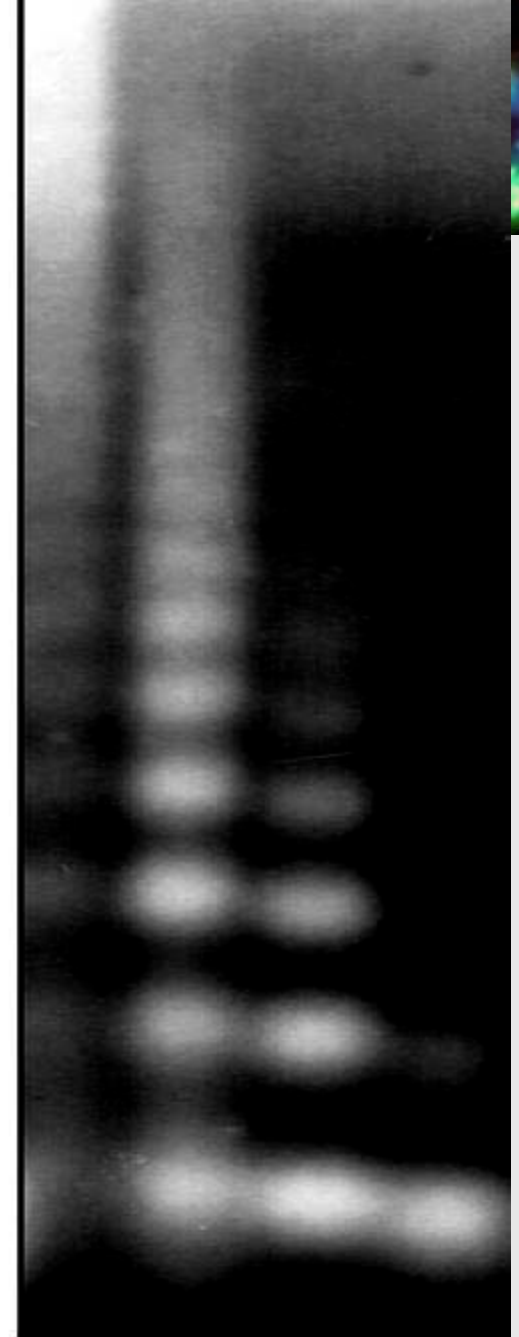


Nucleosomes in Rye

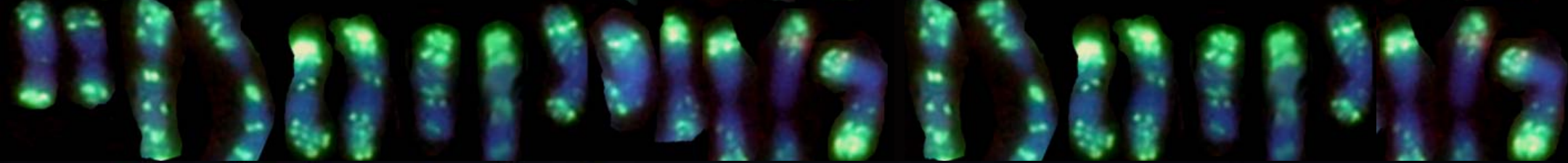
Digest intact chromatin (DNA + histone) with micrococcal nuclease for a few seconds, cutting between the nucleosomes. Then treat with protease and run on agarose gel.

- ↳ Vershinin &
- ↳ Heslop-Harrison

bp
680
510
340
170

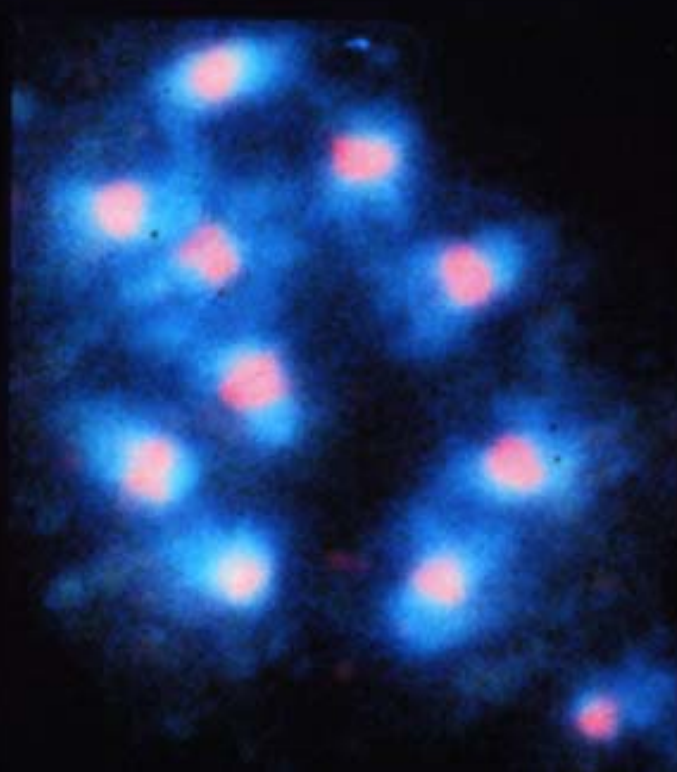
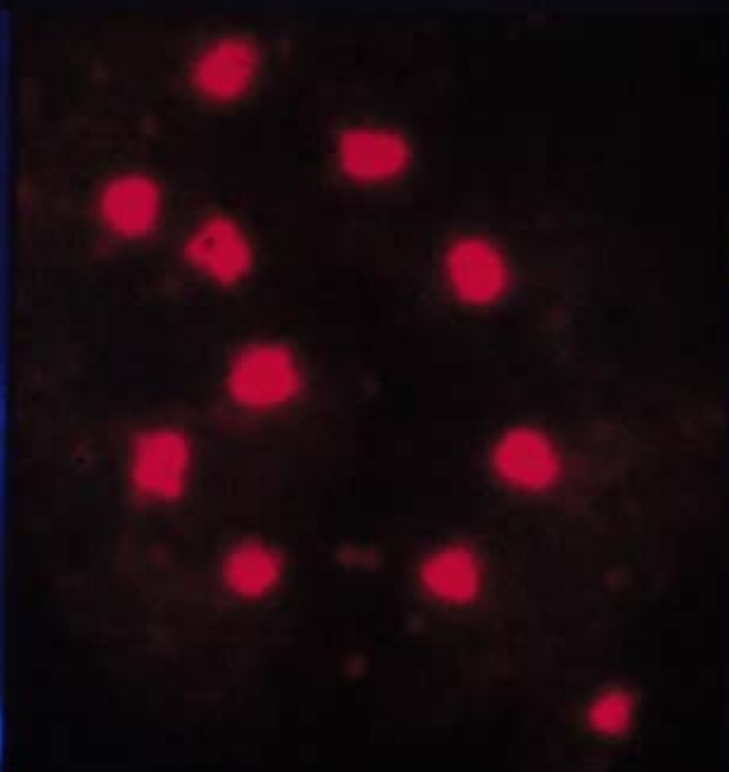
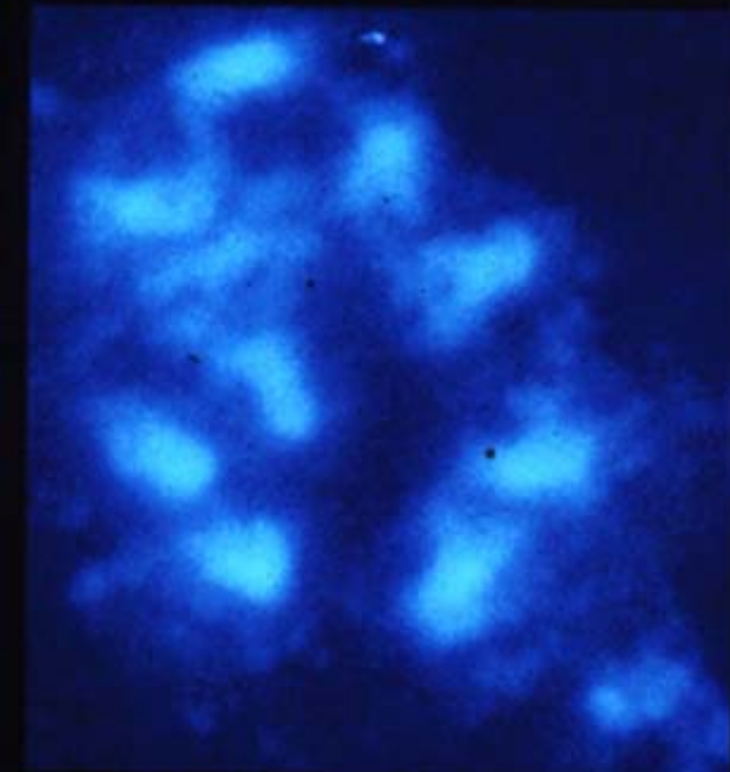


15
45
90
270
seconds



Arabidopsis thaliana

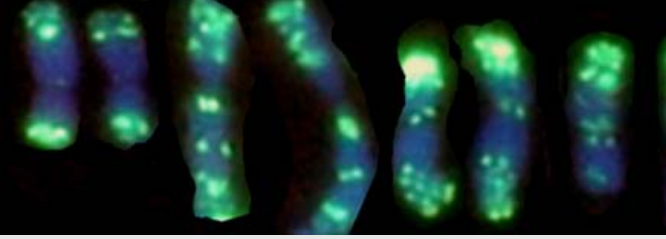
In situ hybridization with AtCen



	←Primer site I→				Box A	←Primer site II →			
	1	2	3	4	5	6	7	8	
123456789012345678901234567890123456789012345678901234567890123456789									
AtHR220/2	1: AACCTTCTTCTTGCTTCTCAAAGCTTT	GATGGTT	TAGCC	GAA	GTC	CAATATGAGTCTTT	CTCTTTGT	ATCTTCTAACAAG	-GAAACACTAC
179: ..G.....	GTAT	I	G	AT	A	-...T..C..
357: ..G.....	C	G	T	CT	A	-.....
AtHR220/3	1: ..G.....	G	T	A	AT	A
159: ..G.....	T	G	T	G	AT	A	T.....
337: ..G.....	G	T	TC	AT	T
525: ..G.....	A	G	T	G	GT	A
693: ..G.....
ATAR11	21: ..G.....	G	T	GG	GT	A
1: ..G.....	G	T	GG	GT	A
ATAR12	1: ..G.....	T	GT	I	G	GT	A
ATAR13	1: ..C.....	T	C	CT	G	A	CG
ATAR14	1: ..C.....	GT	C	I	G	A	CG
AtMP	1: T C	C	G	A	CG	G
ATREAL1A	1: ..C.....	C	G	A	CG	G
ATREAL1B	2: T CG	G	C	G	C	A	C
ATREAS1	1: ..G.....	T	C	G	T	A	AT
ATREAS2	1: ..C.....	C	G	A	CGT	G
AtSATDNA2	1: ..C.....	C	G	A	CG	A
AtSATDNA4	361: ..G.....	C	G	GA	CG	A
						400: ..G.....	CG	A

AtHR220/2	1: AACCTTCTTCTTGCTTCTCAAAGCTTT	GATGGTT	TAGCC	GAA	GTC	CAATATGAGTCTTT	GTCTTTGT	ATCTTCTAACAAG	-GAAACACTAC
Aa27	80: ..A.....	TT	GTG	G	G	T	CGGT	GAG
Aa271	80: ..A.....	T	GTG	G	G	T	CGGT	GAG
Aa214	80: T.....	C	-TG	TCCCT	C	T	CGGT
Aa519	80: T.....	T	C	-TG	A-G	T	CGGT
Aa524	80: ..G.....	A	T	CAACC	-G	T	CGGT

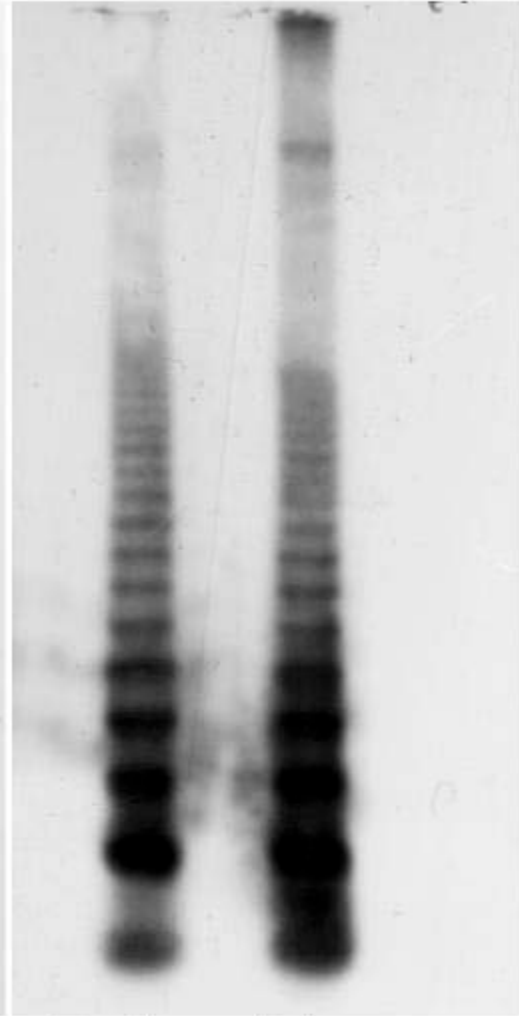
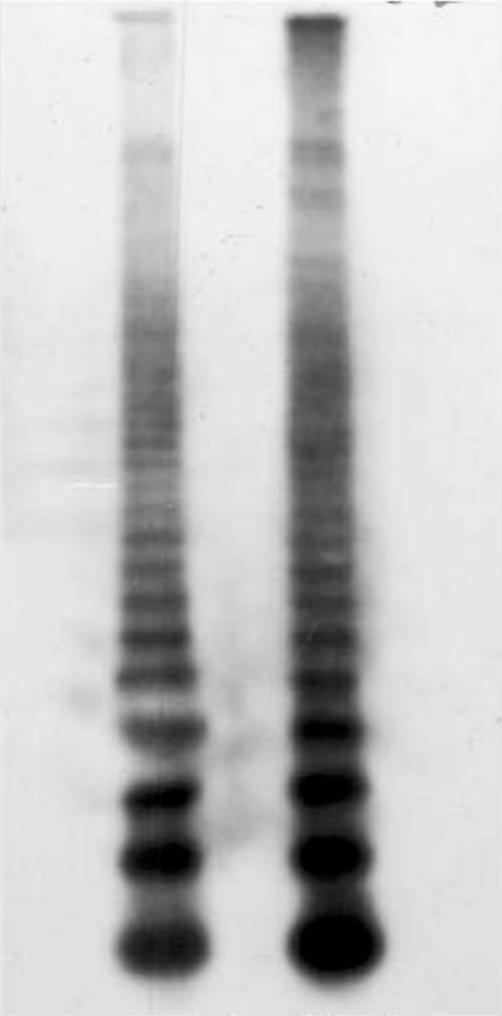
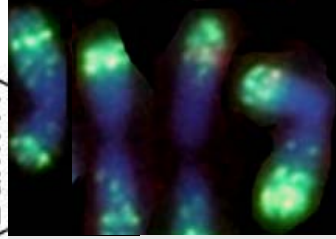
	←Primer site III →			Box B			
	1	1	1	1	1	1	1
9	0	1	2	3	4	5	6
0123456789012345678901234567890							
AtHR220/2	TTAGGCTTGTAGGAGAAGATTGCGGTTTAA	TTCTTATACTCAATCATA	-CACATGACATAAAGTCATATT	CG	-ACTCCAAA	-ACACTCAC	
.....	T	G	TAA	G	A
.....	T	T	G	TAA	G
AtHR220/3	T	G	TAA	G
.....	T	G	TAA	A	T
.....	T	G	TAA	G	T
.....	G	TAA	A



A. thaliana
A. griffithiana
A. suecica
A. pumila
A. arenosa
B. nigra

A. thaliana
A. griffithiana
A. suecica
A. pumila
A. arenosa
B. nigra

A. griffithiana
(*BamH1*)



A. griffithiana

A. pumila

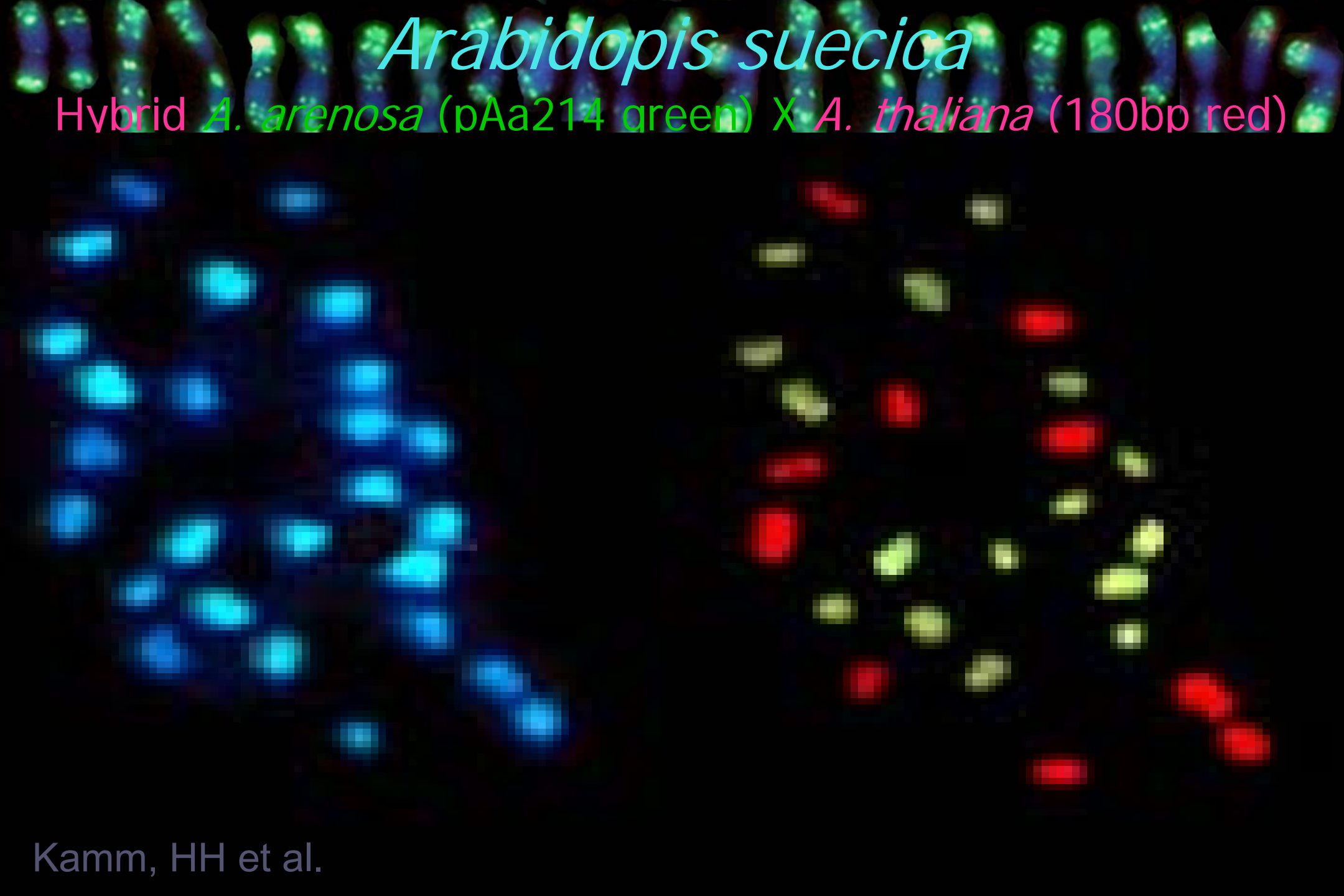
A. grif

HaellI digests; Probe origin

HH, Brandes
2003

Arabidopsis suecica

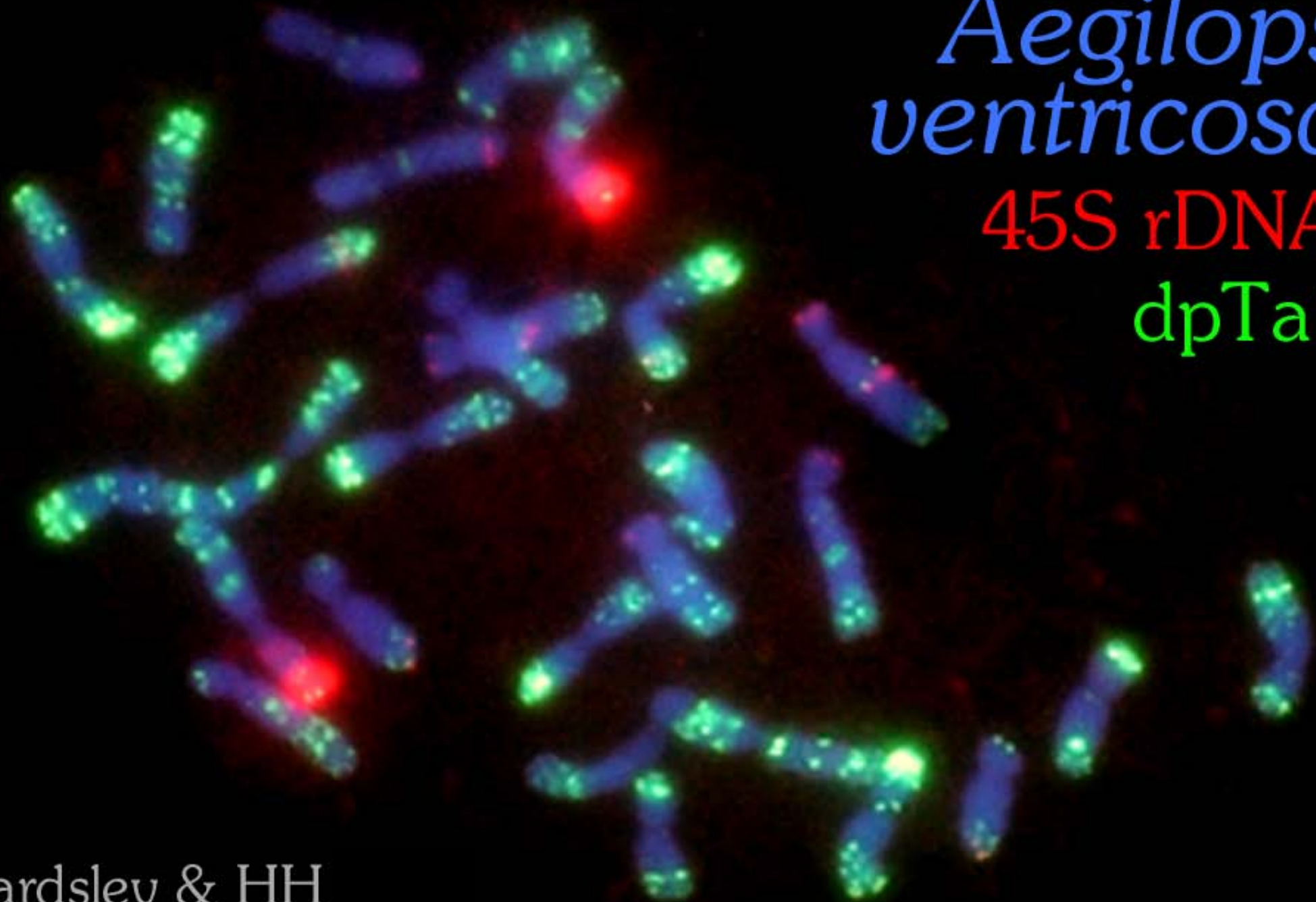
Hybrid *A. arenosa* (pAa214 green) X *A. thaliana* (180bp red)



*Aegilops
ventricosa*

45S rDNA

dpTa1



Wheat Species Evolution

Common Ancestral species
 $2n=2x=14$

Triticum tauschii
 $2n=2x=14$
DD

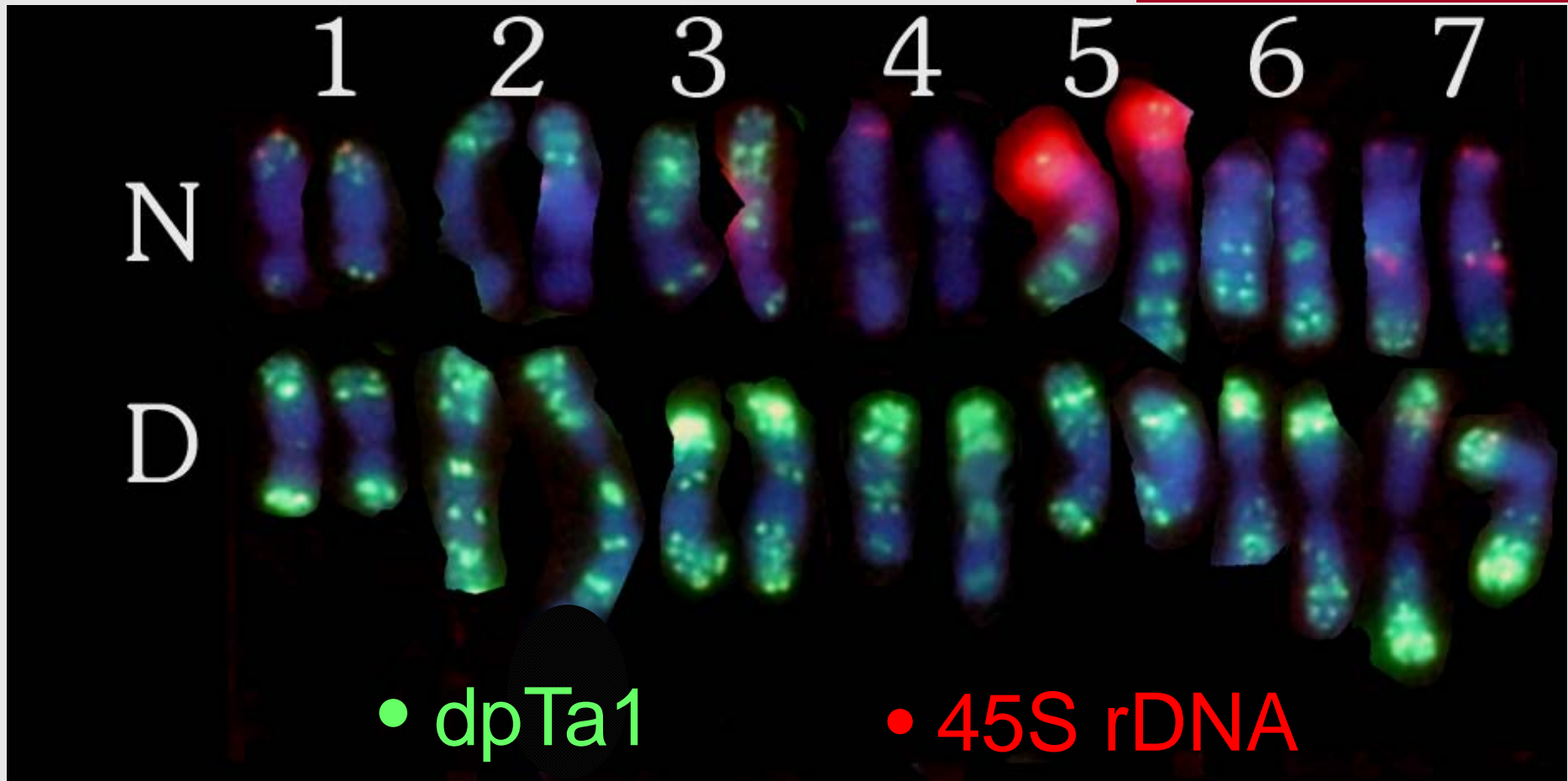
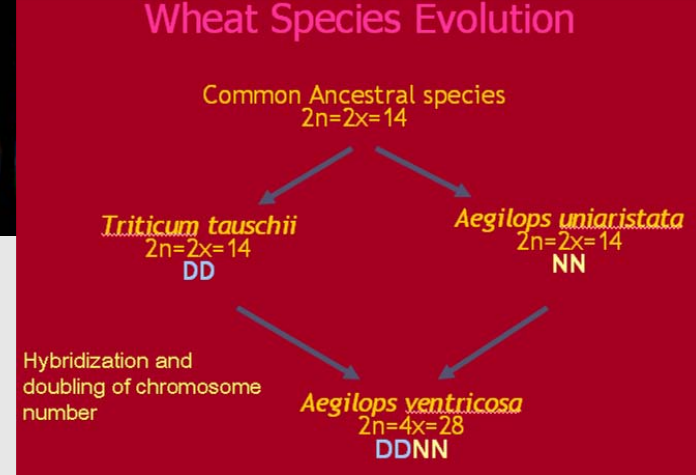
Aegilops uniaristata
 $2n=2x=14$
NN

Hybridization and
doubling of chromosome
number

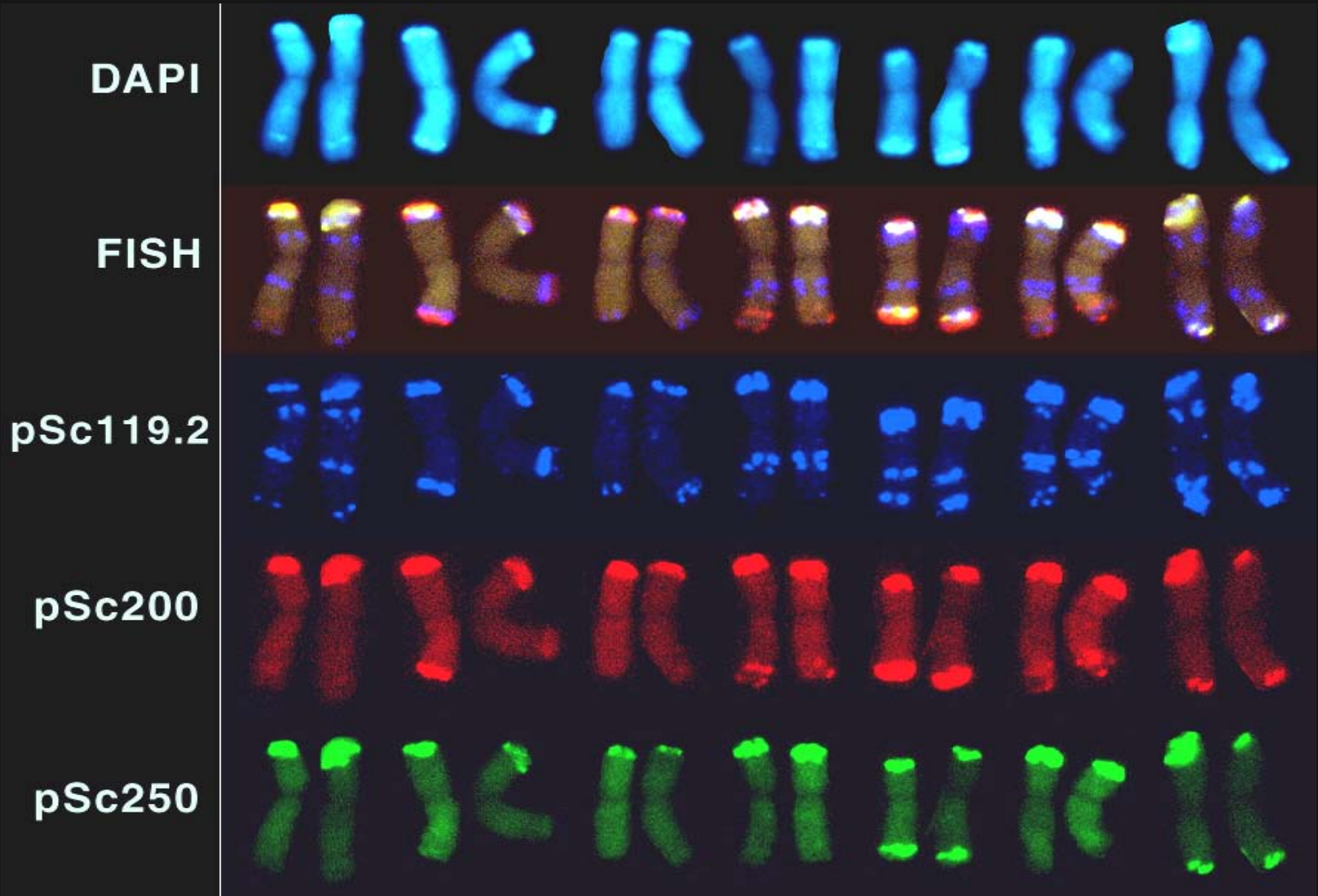
Aegilops ventricosa
 $2n=4x=28$
DDNN

Differences between genomes

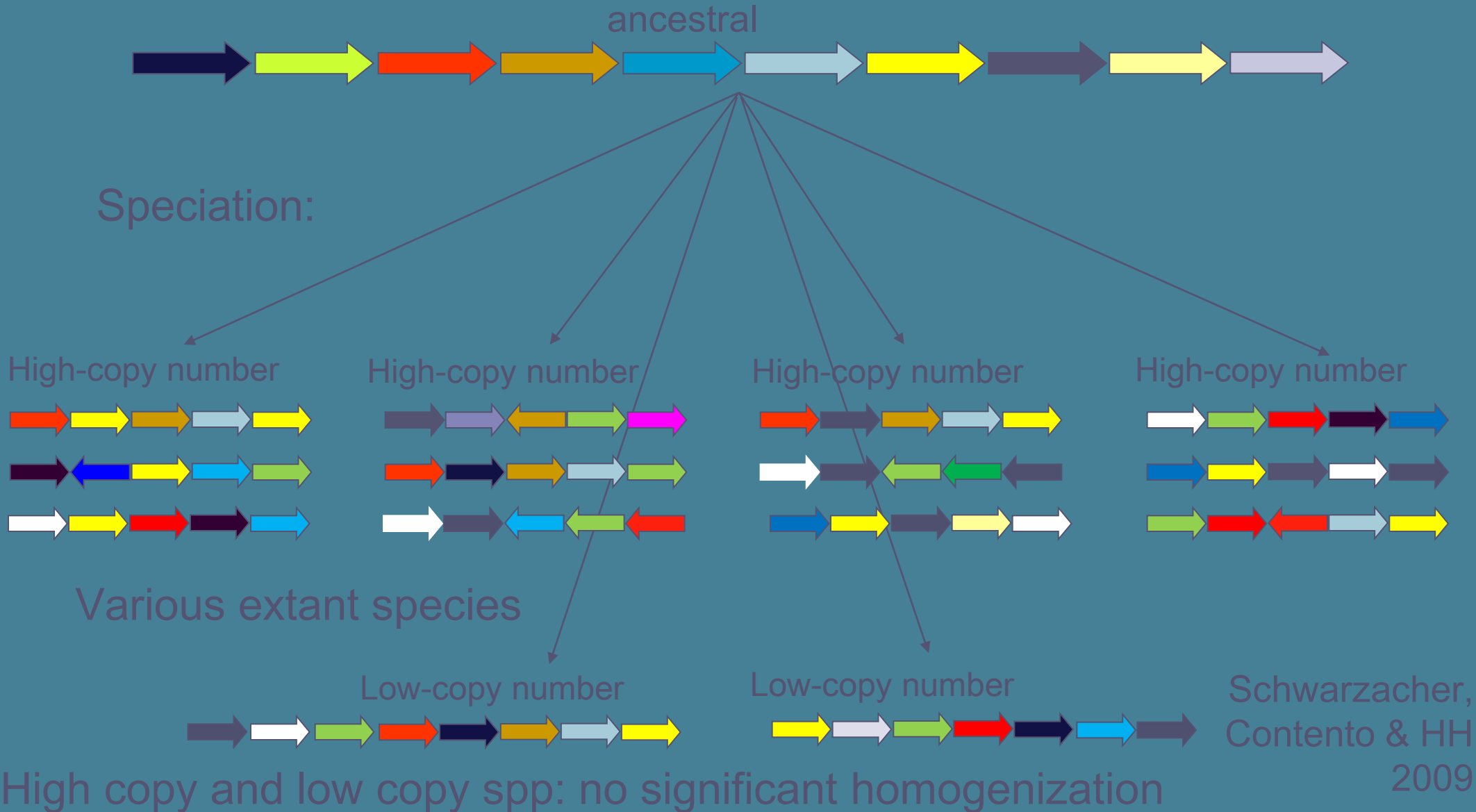
Major differences in the nature and amount of repetitive DNA: new & old



Rye: subtelomeric sequences

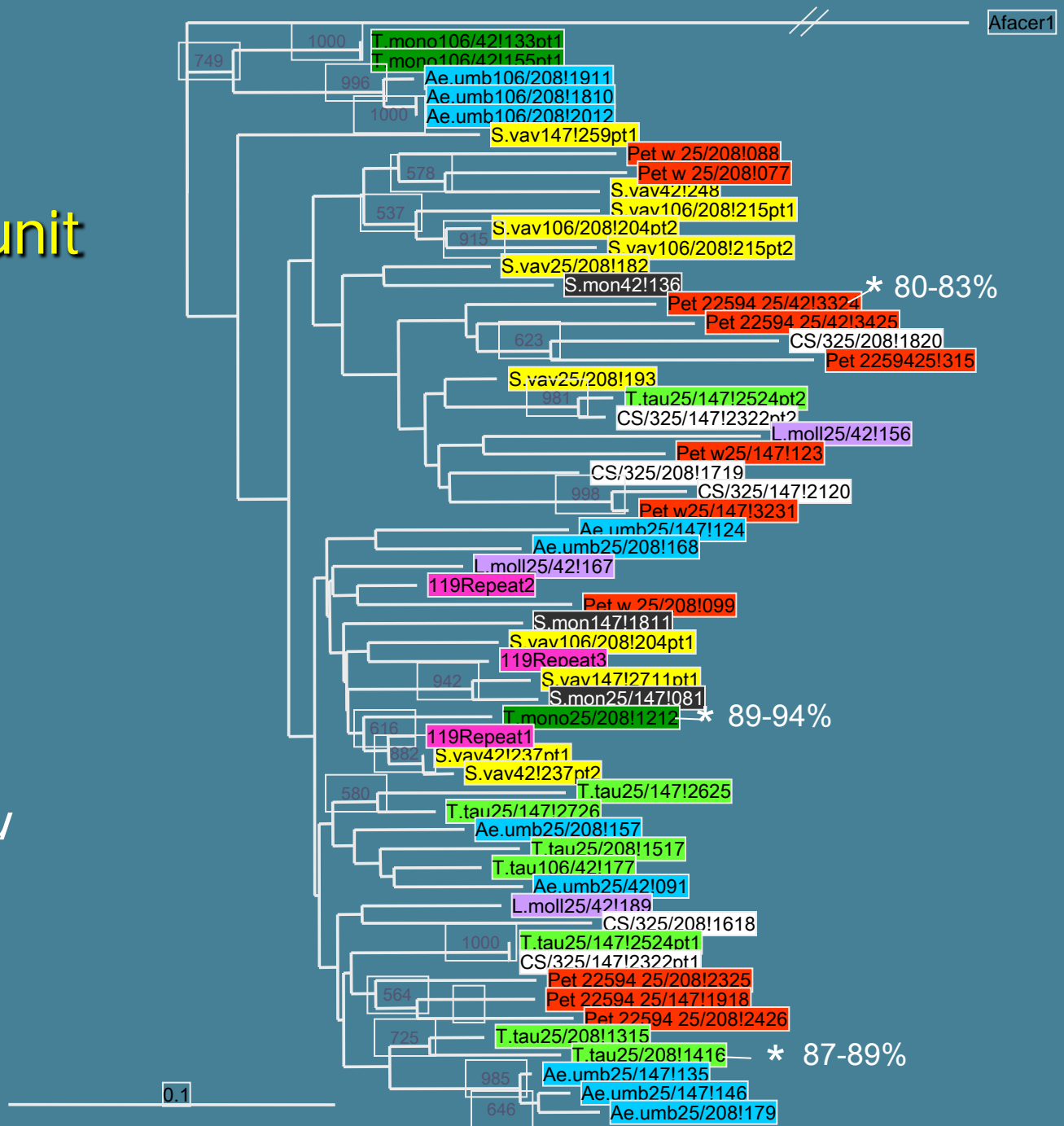


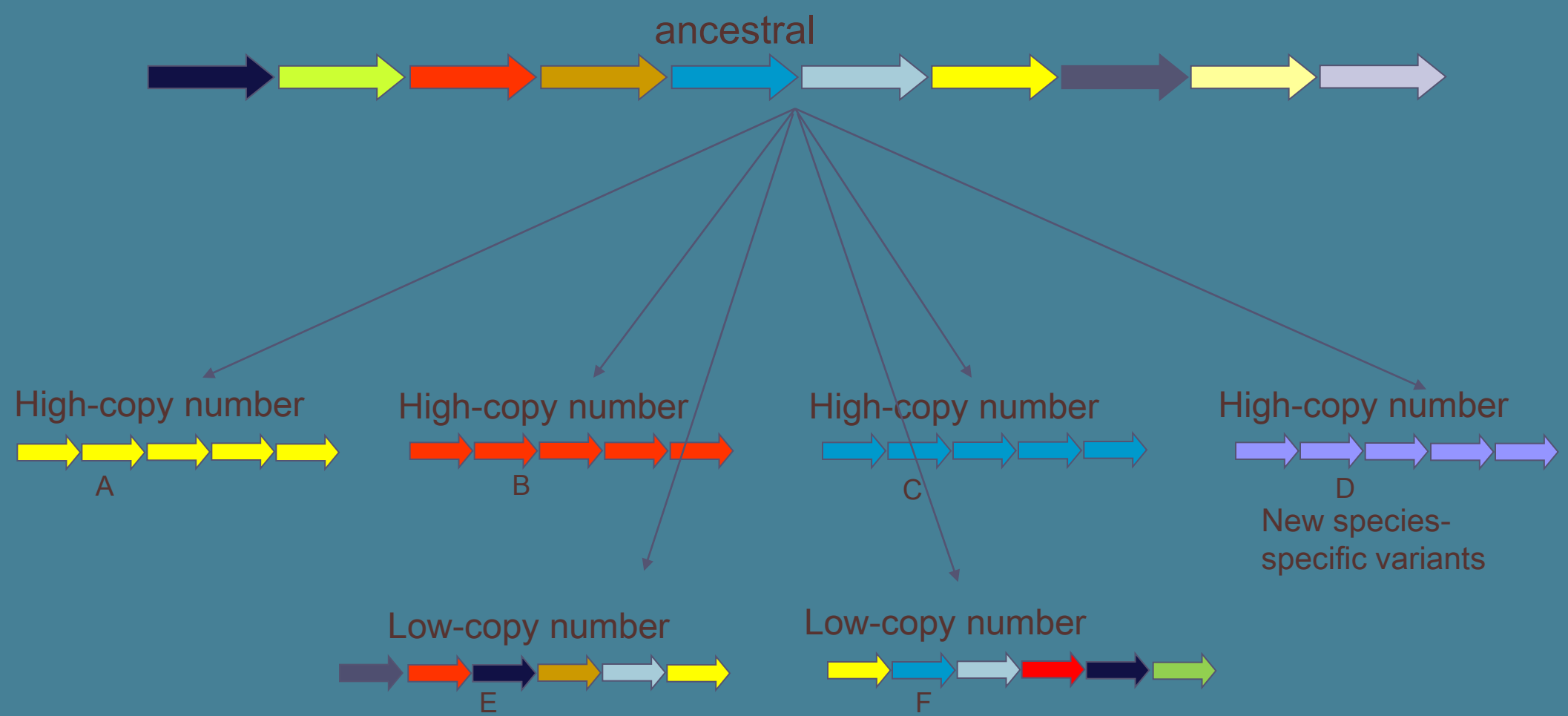
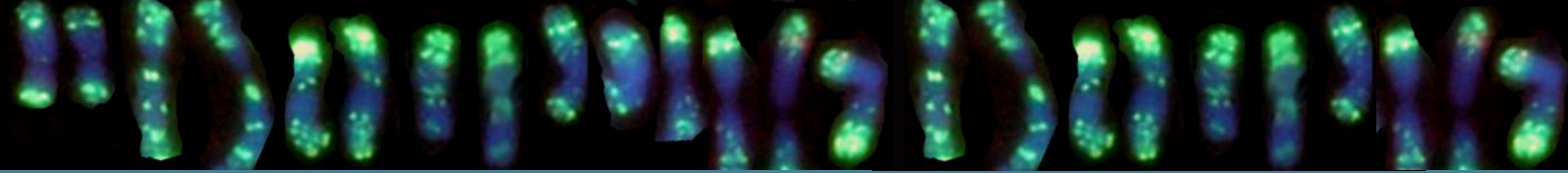
119.2 and the 120bp repeat family in cereals



120bp repeat unit family in *Triticum*, *Aegilops* and *Secale* species

* Sequences used for *in situ* hybridization





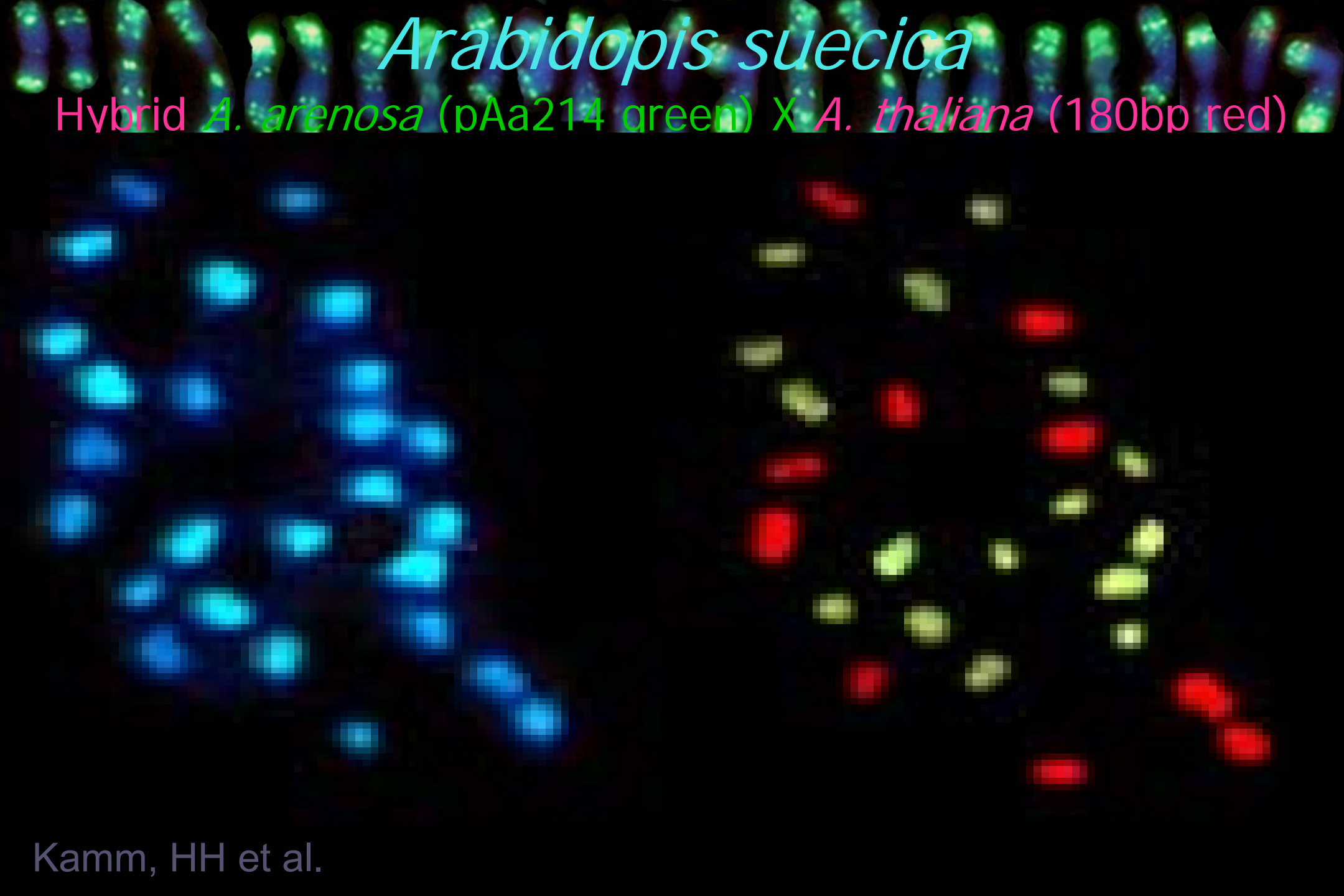
High copy spp: homogenized old (ABC) or new (D) variants

Low copy spp: most old variants in low copy number (EF)

See Kuhn, HH et al. 2009. Heredity & 2008. Chr. Res.

Arabidopsis suecica

Hybrid *A. arenosa* (pAa214 green) X *A. thaliana* (180bp red)



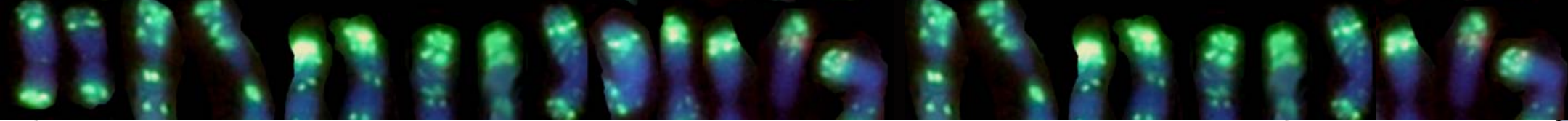
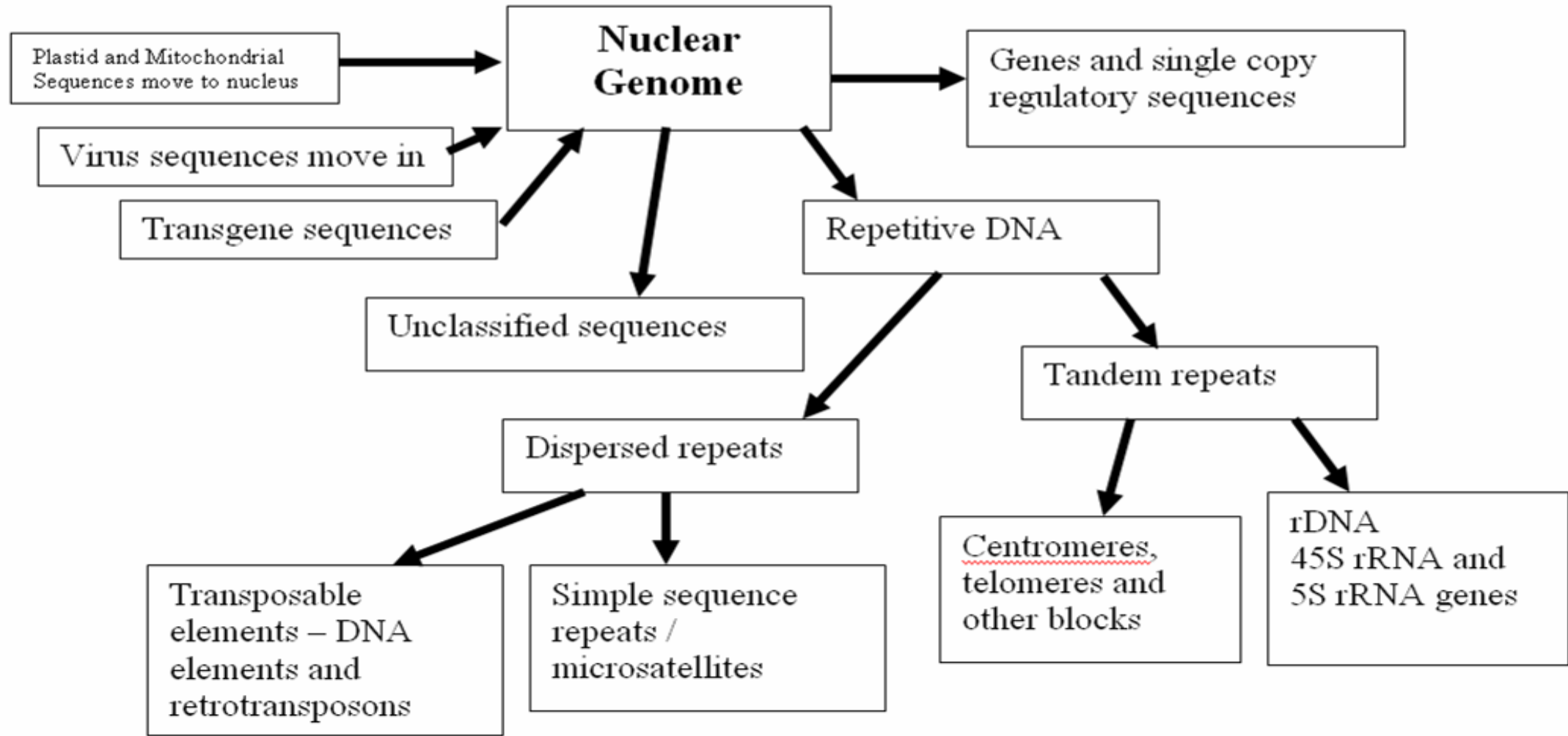
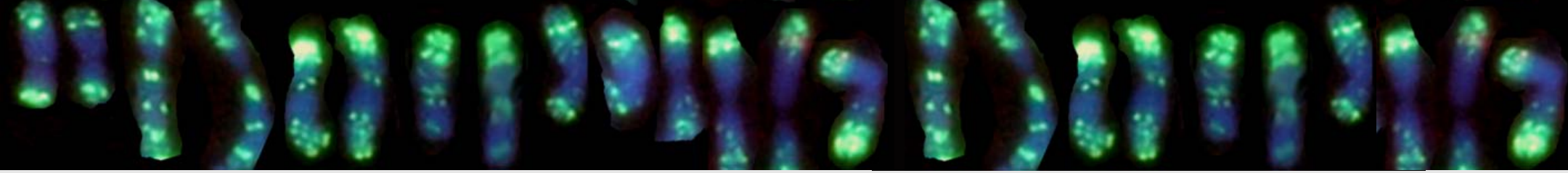


Figure 2. Components of the Nuclear Genome and their Relationships

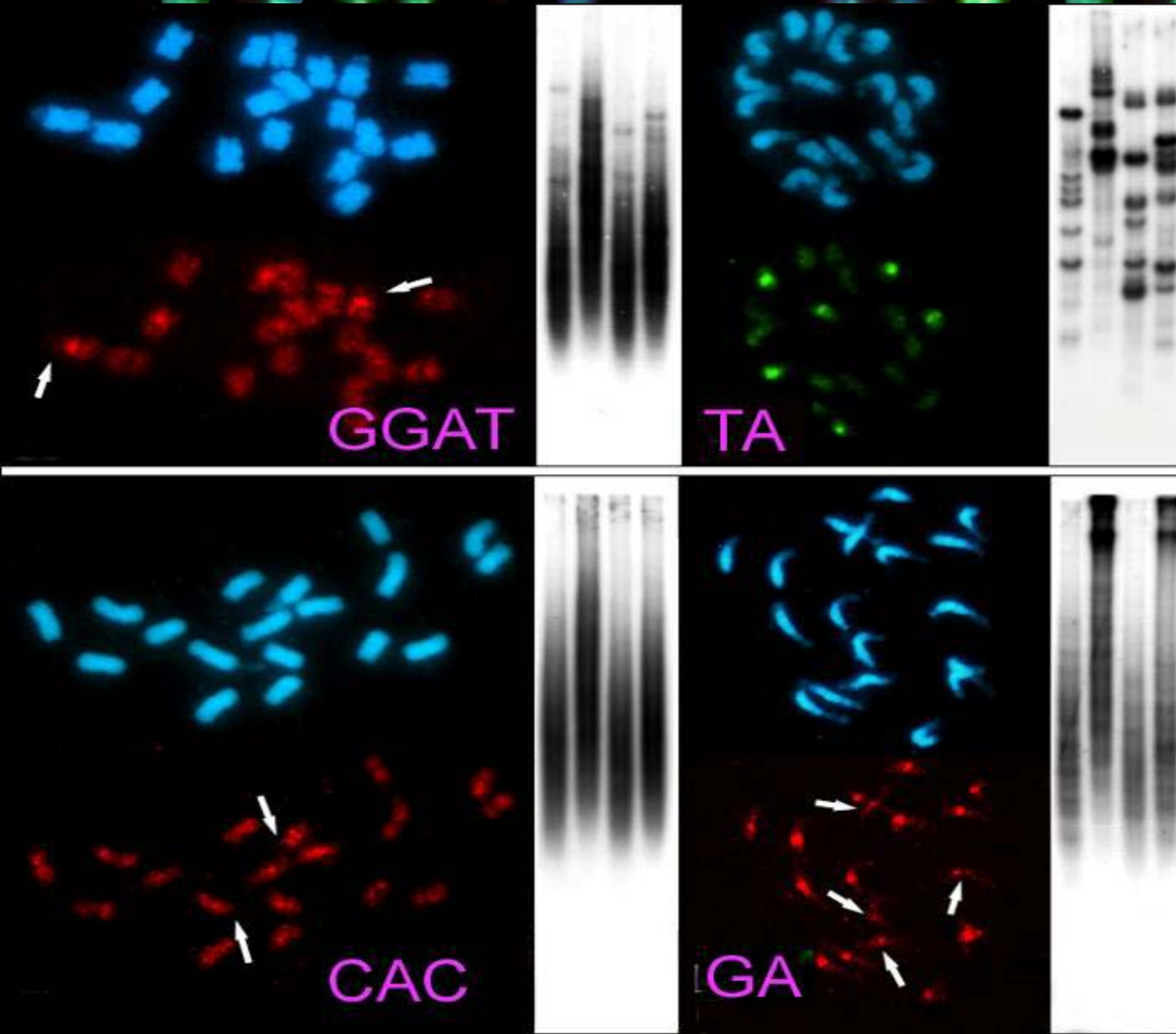




Simple sequence repeats

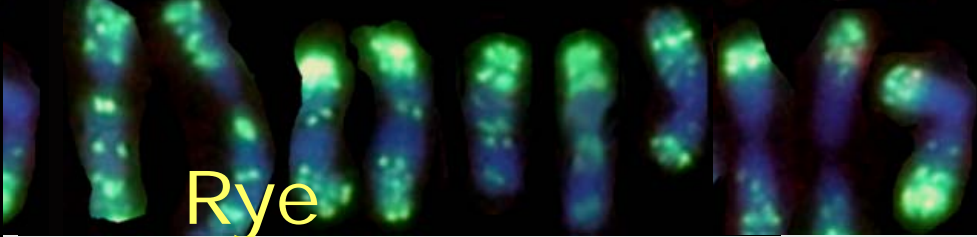
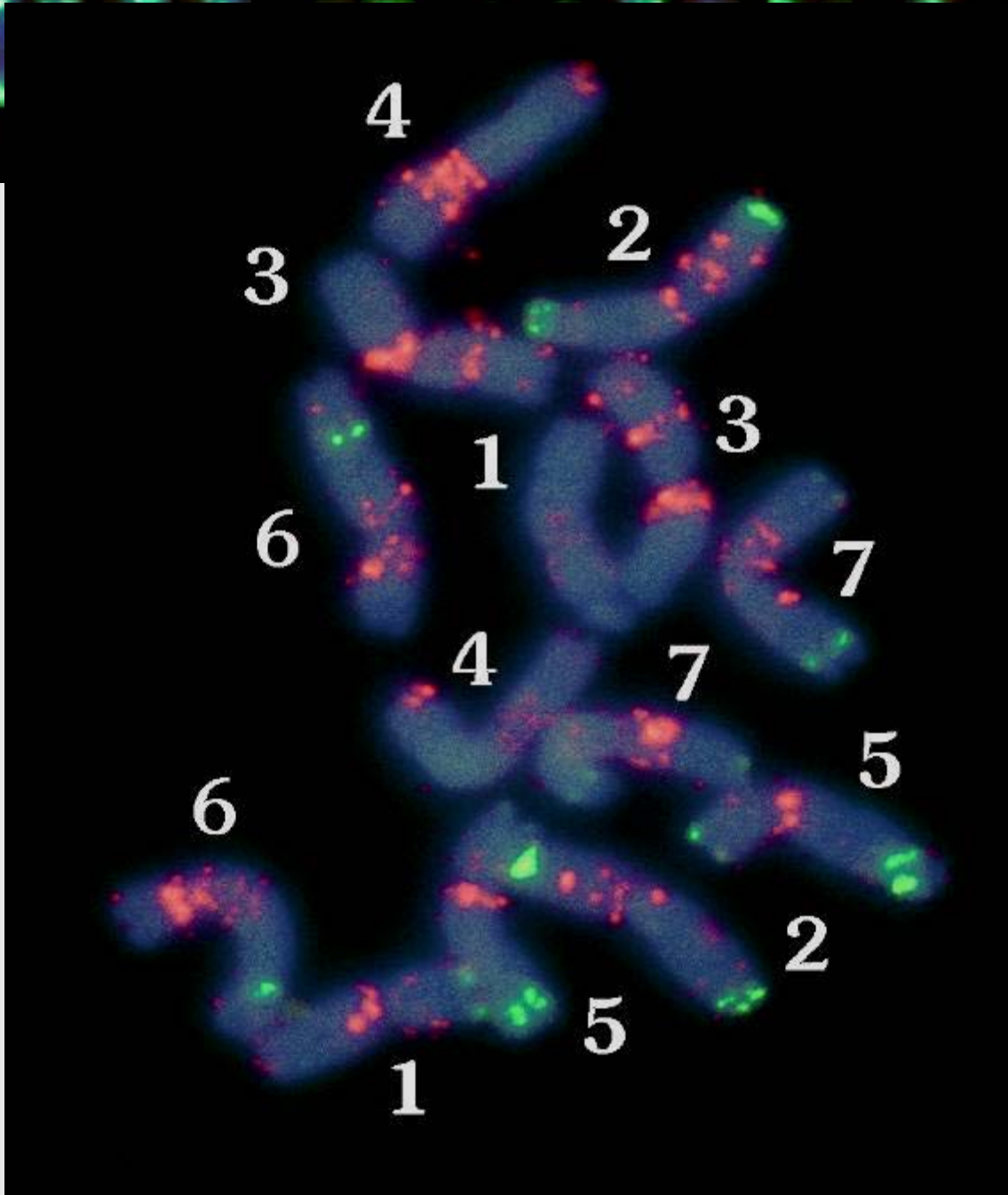
- ☛ GGCTACGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
GAGAGATGGTCGTAATG
- ☛ Flanked by unique sequences (SSR/microsatellite markers) or
- ☛ Part of other repetitive elements
- ☛ Dispersed OR clustered in genome
- ☛ SSR markers are dispersed!

Simple Sequence Repeats



Sugar beet:
Characteristic
organization of
each motif

Schmidt, HH et al.



Rye

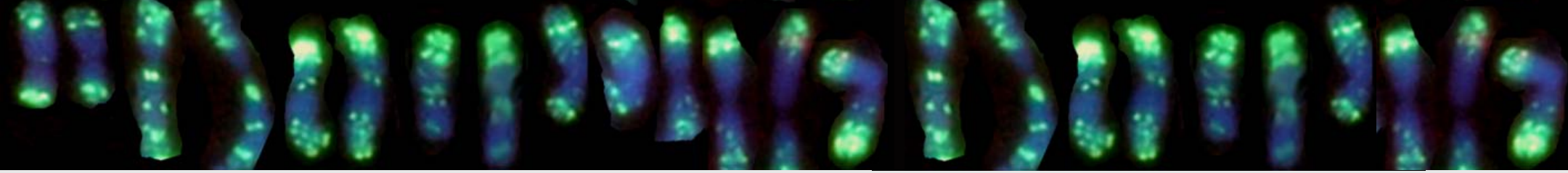
Secale cereale

$2n=14$

DAPI

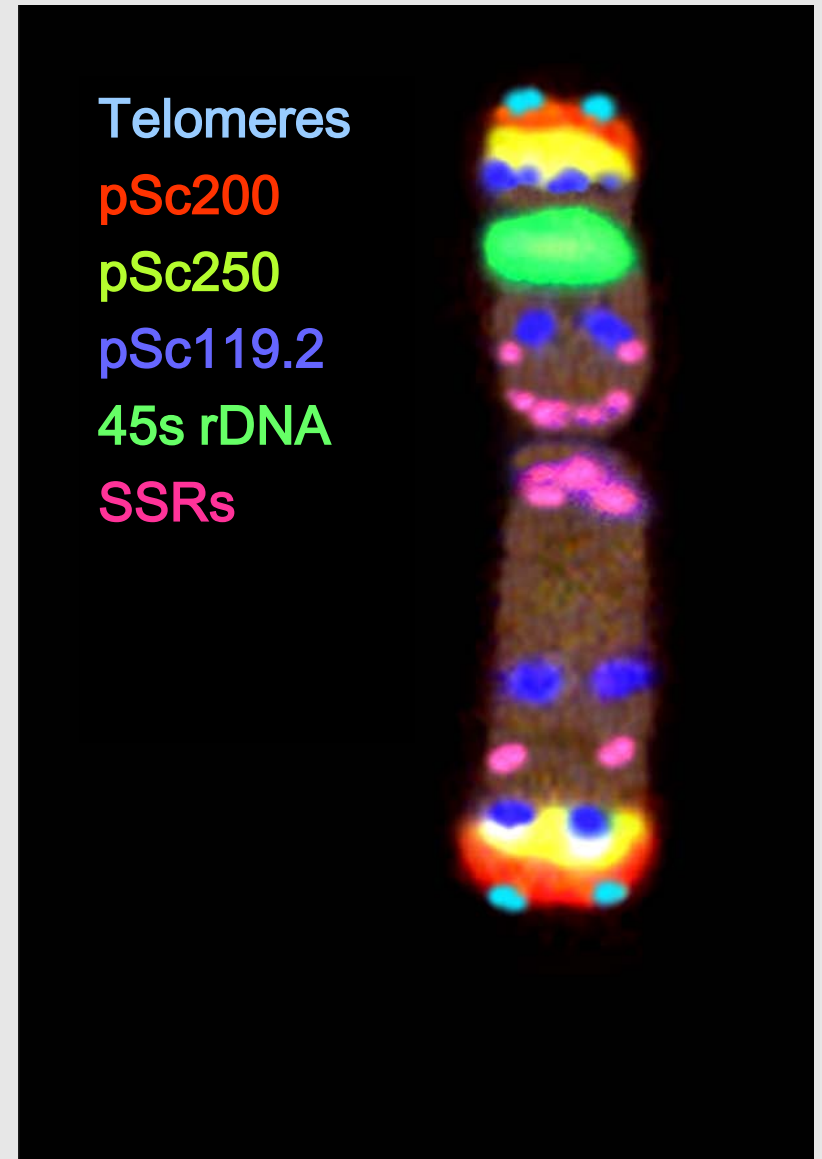
FITC/Alexa 488

Cy3/Alexa 594

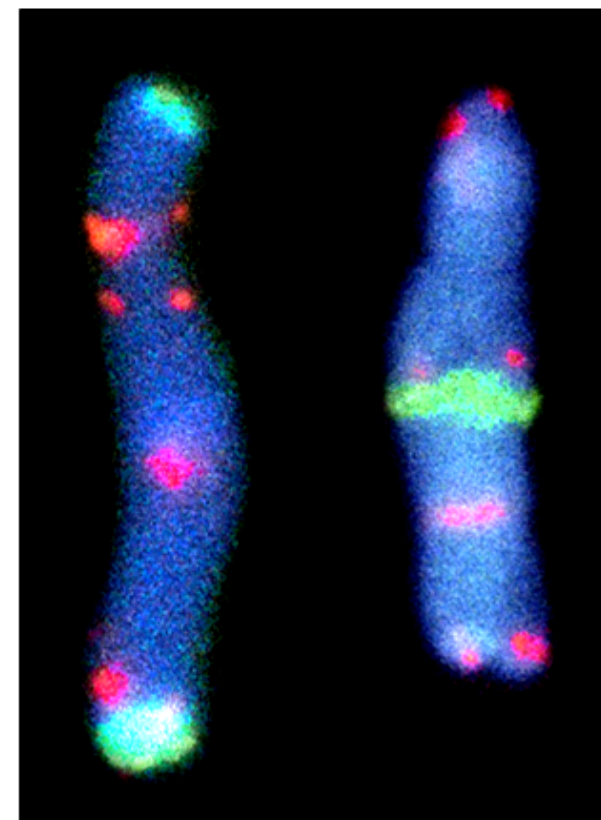
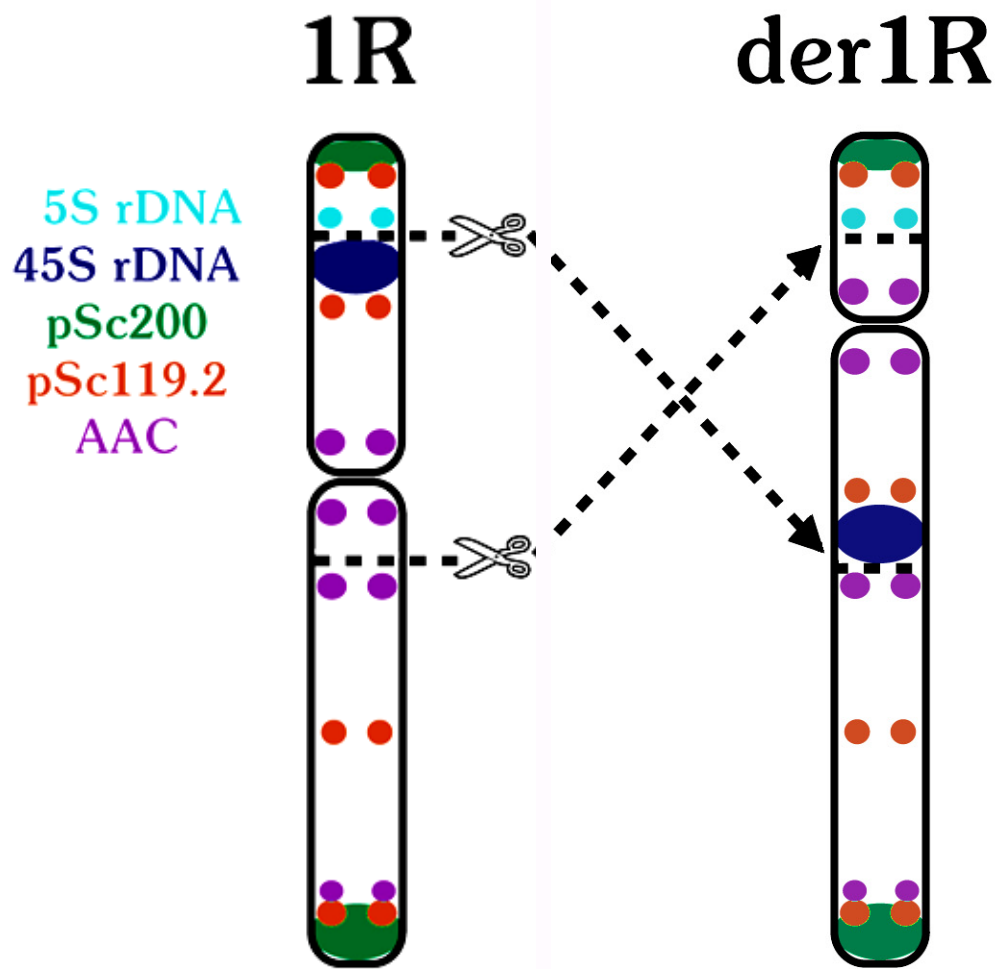


Chromosome model

1R of rye



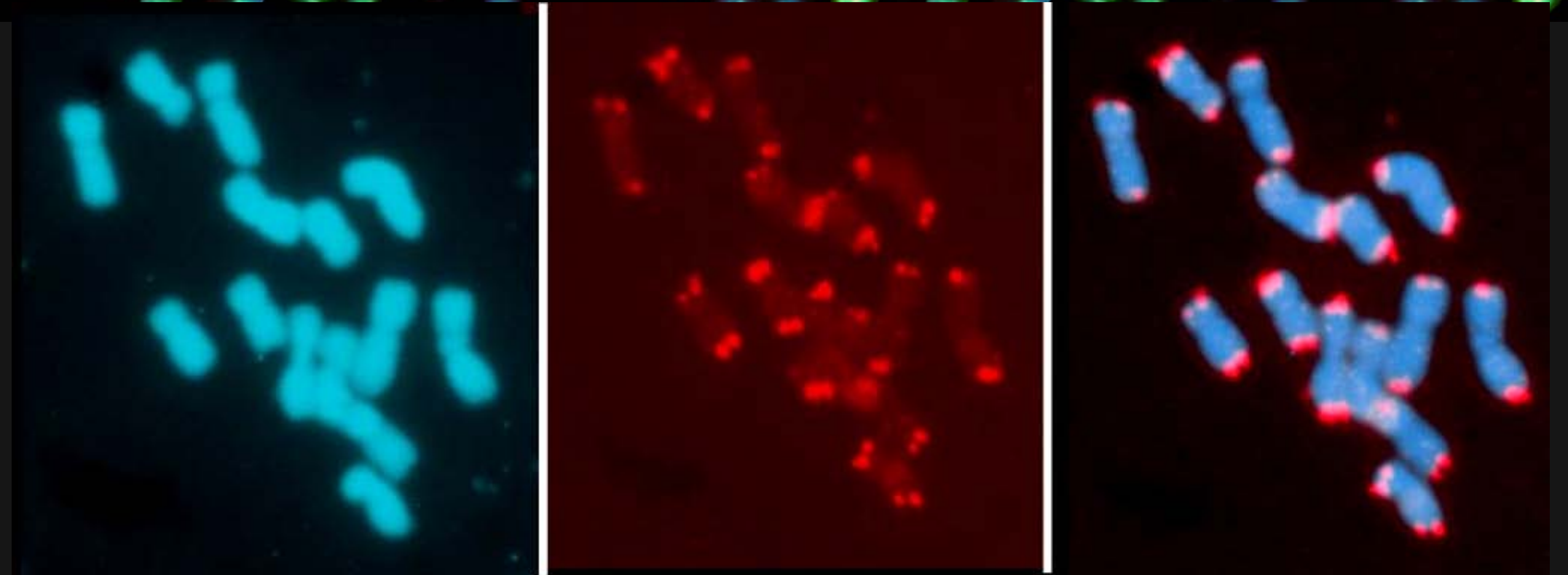
Derivative chromosome 1R of Lines 7-102 and 7-169



AAC
pSc200

pSc119.2
pTa71

Telomere (TTTAGGG)_n



- ☛ Universal in eukaryotes with only a few exceptions
- ☛ Dynamic
- ☛ Number of repeats varies: tissue, age and chromosome
- ☛ Added by telomerase

Oil Palm



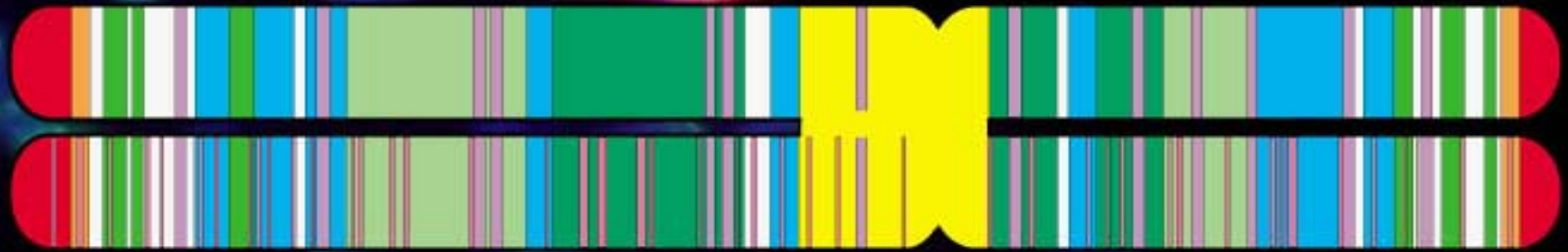
32 chromosomes DAPI;

TTTAGGG telomere;

45S rDNA (1 major pair + minor)

5S rDNA (1 major + minor)

The Linear Chromosome



Tandem repeats



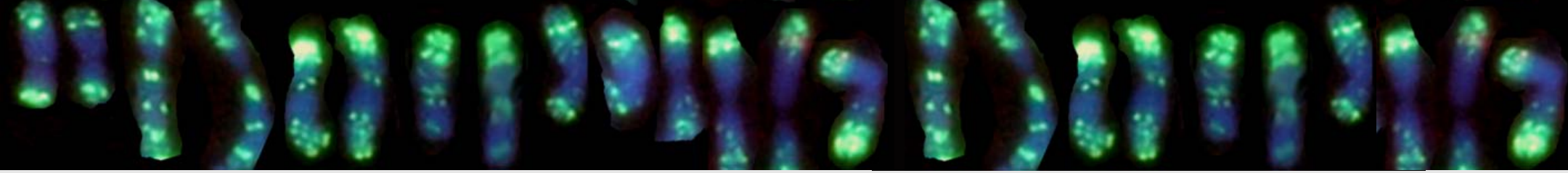
Retroelements
Simple sequence repeats



Terminal repeats



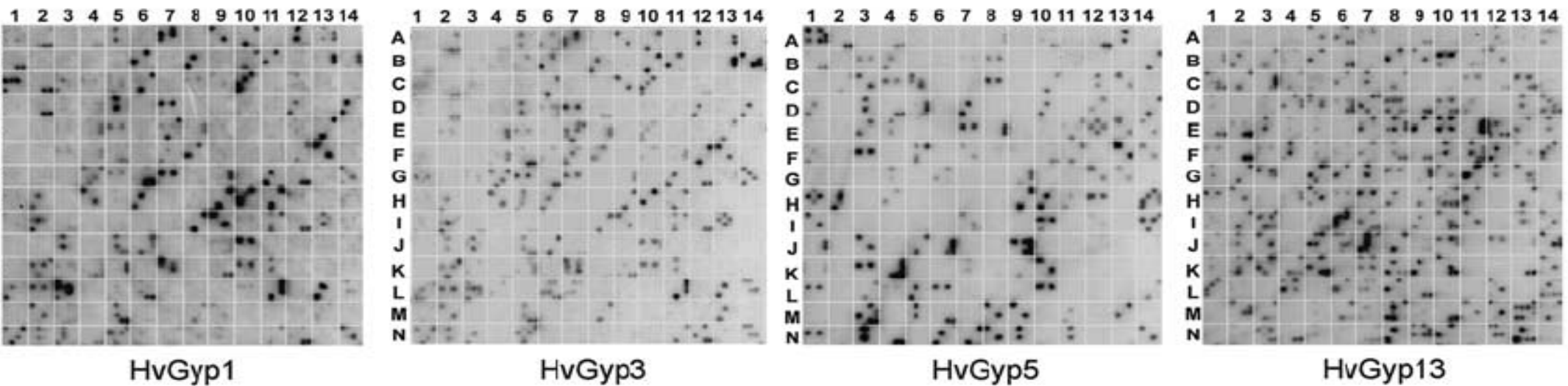
Genes



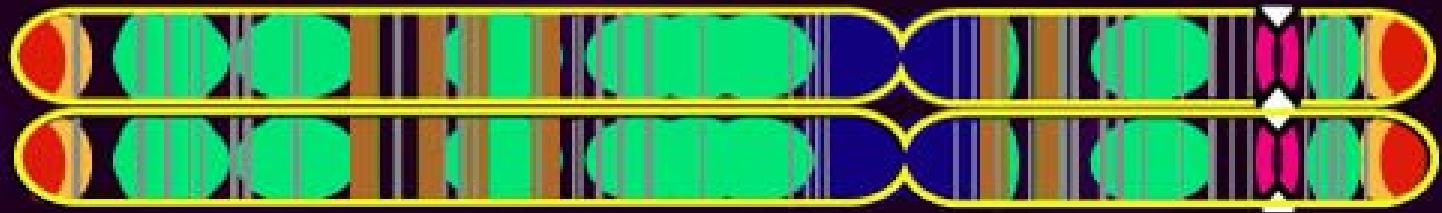
Repetitive Sequences

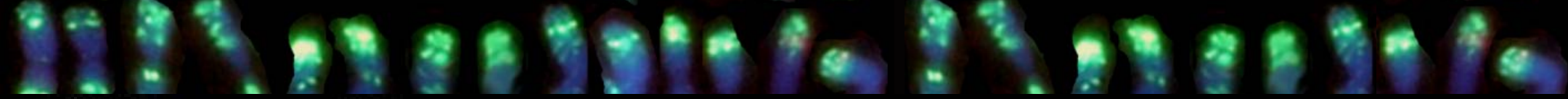
- ☞ The majority of the genomic DNA in most species (95% sometimes)
- ☞ Tandem Repeats
- ☞ Simple Sequence Repeats
- ☞ Dispersed Repeats
- ☞ Functional Repeats
- ☞ Retroelements

Retroelement abundance and diversity in barley

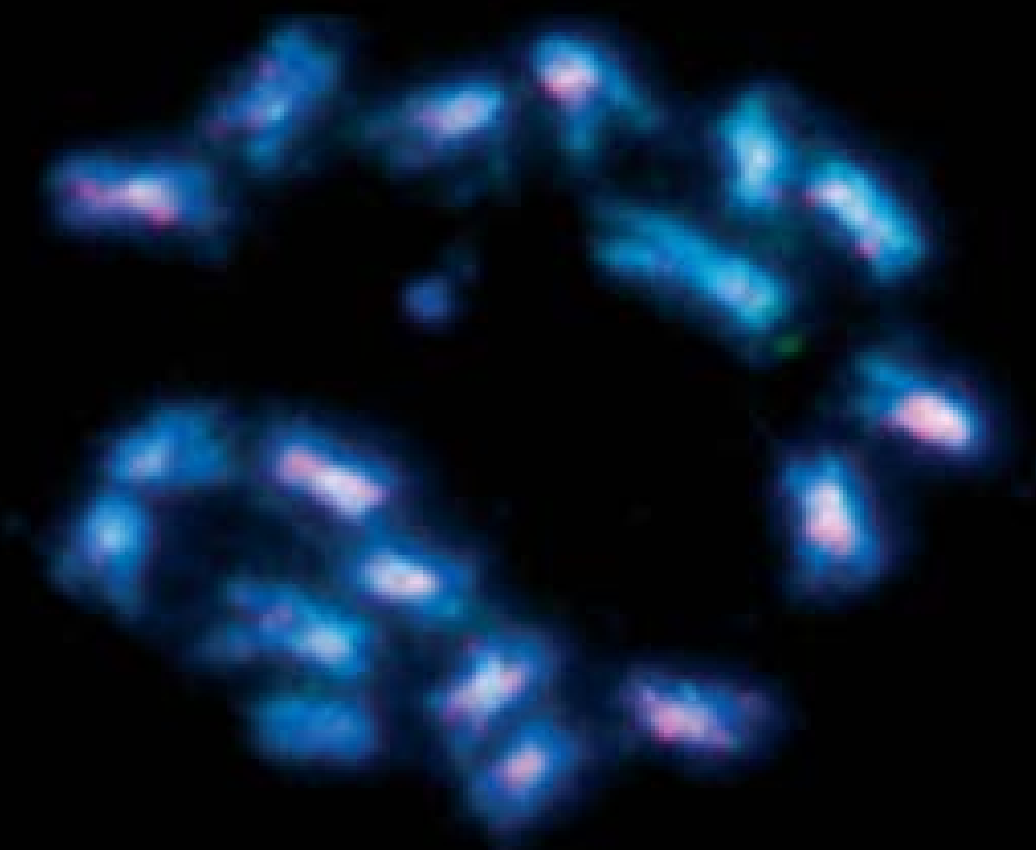
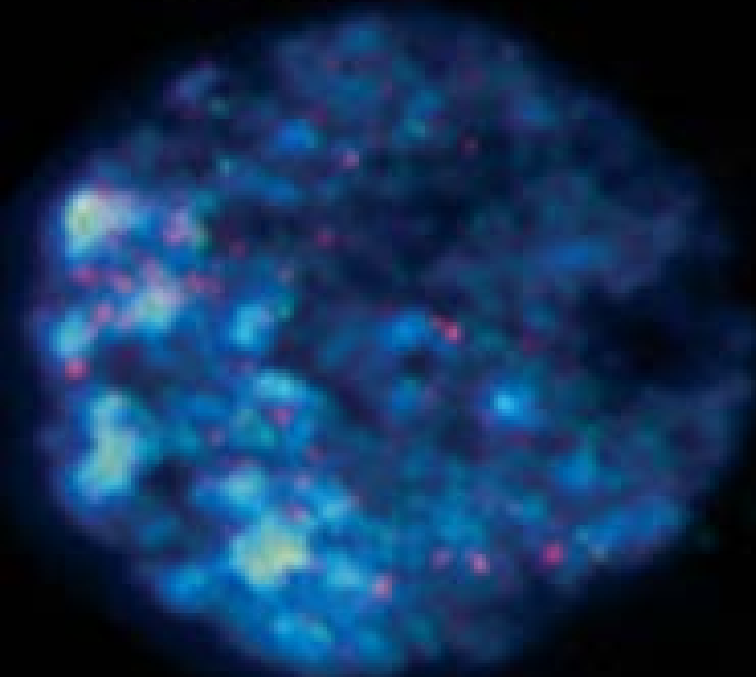


Gypsy elements are present in 25% of all BAC clones





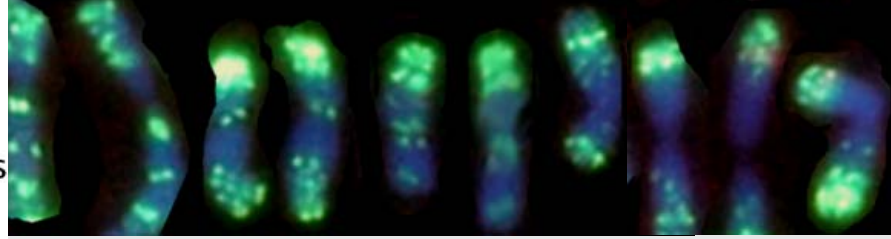
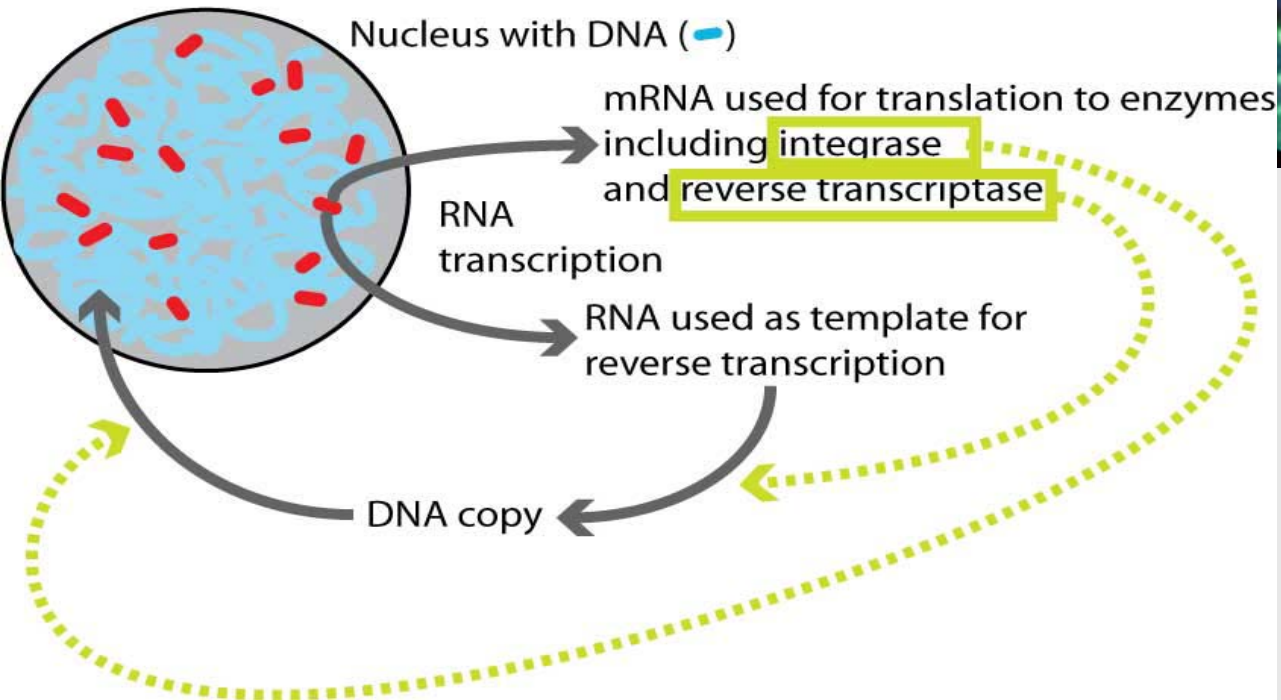
PLANT
MOLECULAR
BIOLOGY



 International Society for
Plant Molecular Biology
 Springer

Retroelements in Brassica

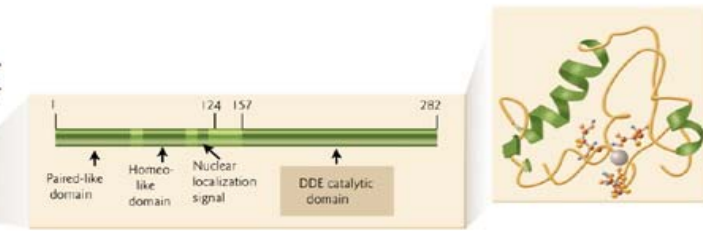
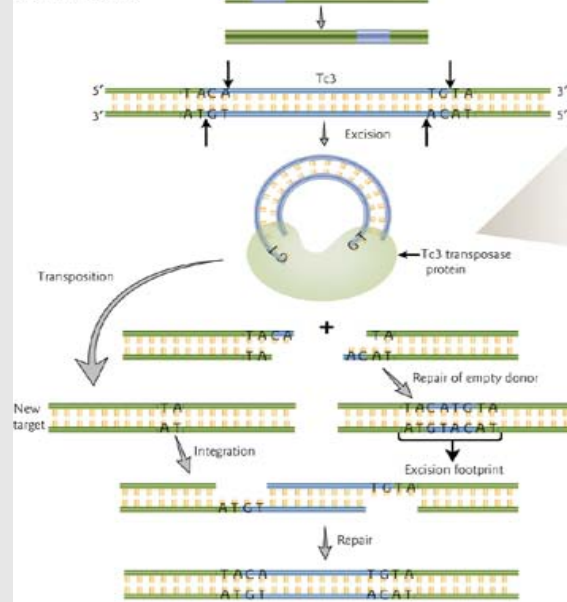
Retrotransposons (-): The transposition cycle



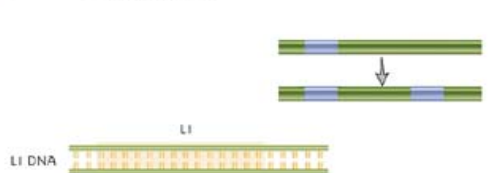
DNA transposons
Class II transposable elements
Cut-and-paste

Retrotransposons
Class I transposable elements
RNA intermediate

(A) Cut & Paste



(B) Copy & Paste



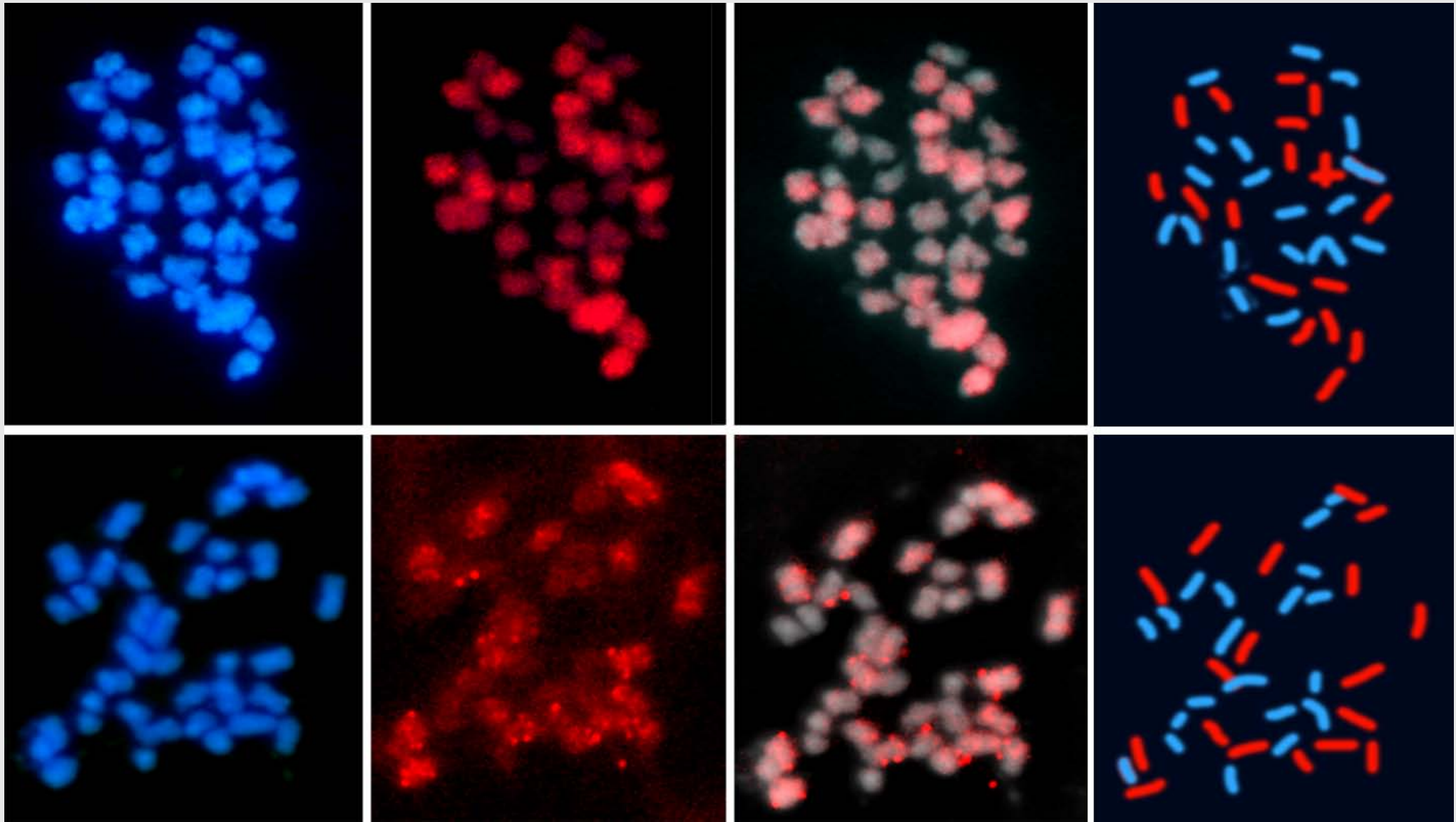
Genome Specificity of a CACTA (*En/Spm*) Transposon

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

B. oleracea (CC, $2n=2x=18$)

B. rapa (AA, $2n=2x=20$)

Alix & HH 2008





LINE Retrotransposon
(non-LTR Retrotransposon)



Gypsy
(LTR Retrotransposon)



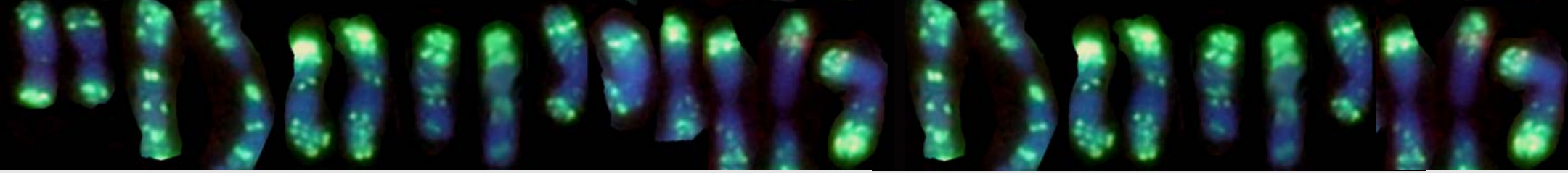
Copia
(LTR Retrotransposon)



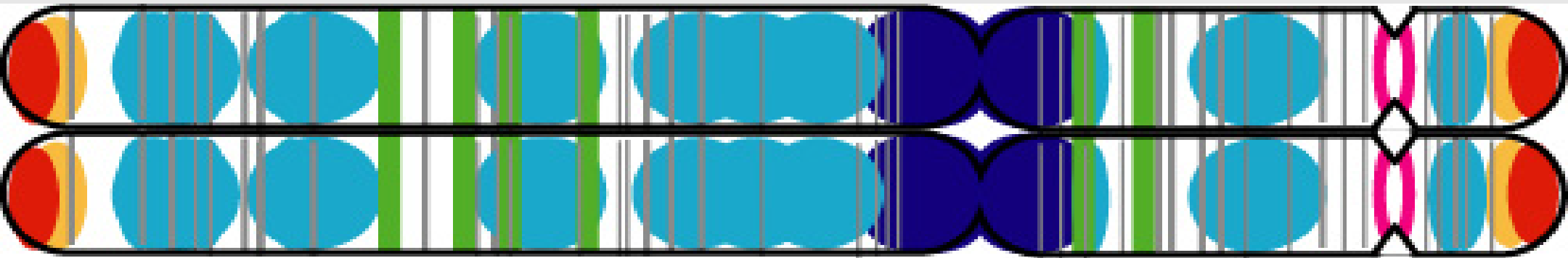
Retrovirus

Common structure of Retroelements







- gag – core particle component
- en – endonuclease
- rt – reverse transcriptase
- LTR – long terminal repeat
- env – envelope glycoprotein



The Chromosome Model



Tandem repeats with known function

-  Telomeric
-  Centromeric
-  rDNA
-  Intercalary tandem repeat families
-  Dispersed repeats including retroelements and SSRs
-  Genes and regulatory sequences

500 nm





Bos taurus taurus vs *Bos taurus indicus*:

$2n=60$, XY

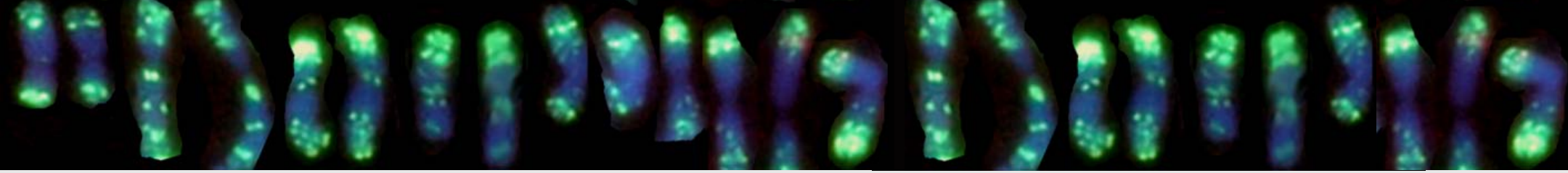
But: *B. taurus* submetacentric Y

B. indicus acrocentric Y

Bovidae

Cattle, sheep, goats, and antelopes

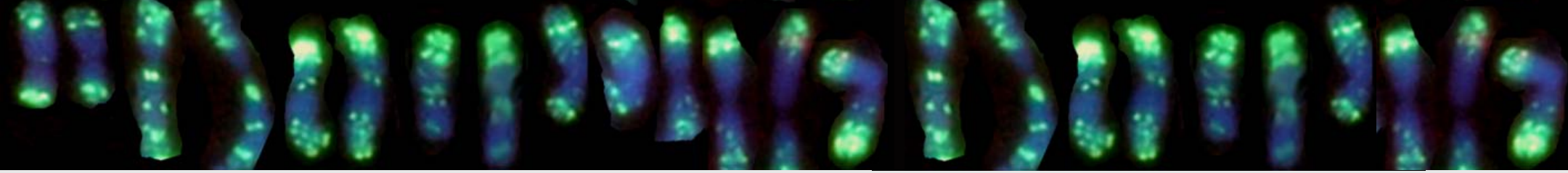




Bovidae – Family

☞ Mammals

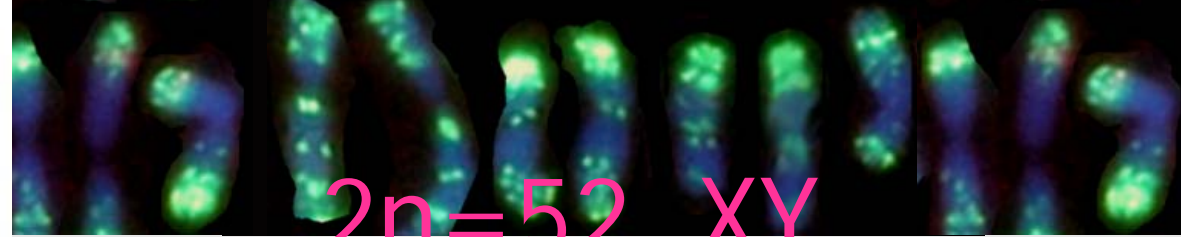
- Order Artiodactyla (=Even-toed ungulates)
- 3 groups: the Suiformes (pigs, peccaries, hippopotamuses), Tylopoda (camels, llamas) and Ruminantia (cattle, goats, sheep, deer, antelopes, giraffes)
- 9 families (13 tribes) including Bovidinae
 - Family Bovidae
 - c. 137 species
 - Last species (new genus) discovered in 1992



Bovidae family

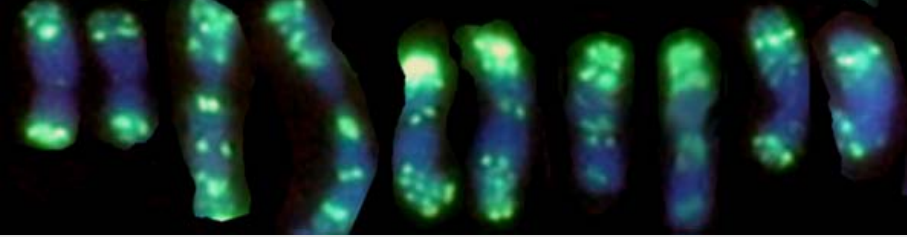
- 2n ranges from 30 to 60 in different species

- (Galagher & Womak 1992)

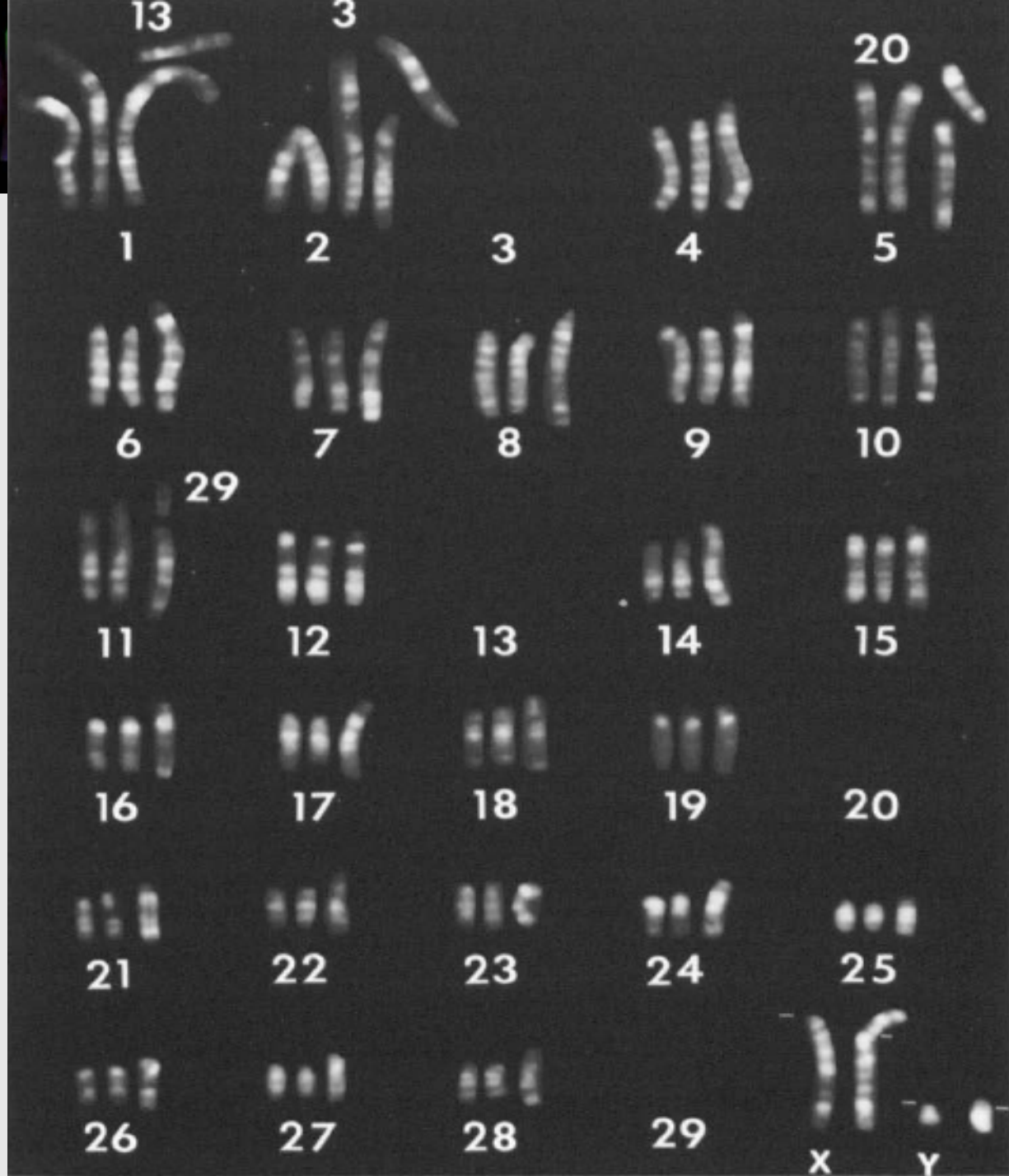


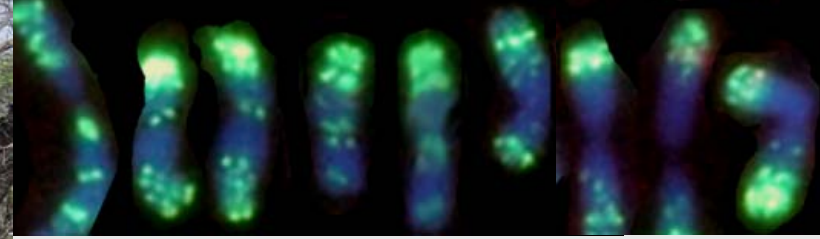
$2n=52, XY$
including 4
bi-armed
chromosomes = 58
autosomal
chromosome arms
+ X, Y

- *Syncerus caffer* (African Buffalo or Cape Buffalo), a bovid from the family of the Bovineae



- Male *Syncerus caffer* QFH band karyotype (left 2 chrs) with cattle chrs to right
- Gallagher & Womak 1992





Tragelaphus strepsiceros or greater kudu

$2n=31$, X1 X2 Y
26 biarmed
chromosomes, three
acrocentric
chromosomes (inc.
X1), acrocentric X
and a biarmed Y

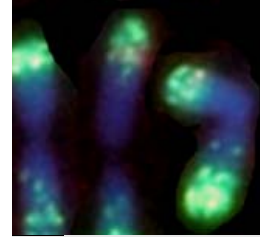
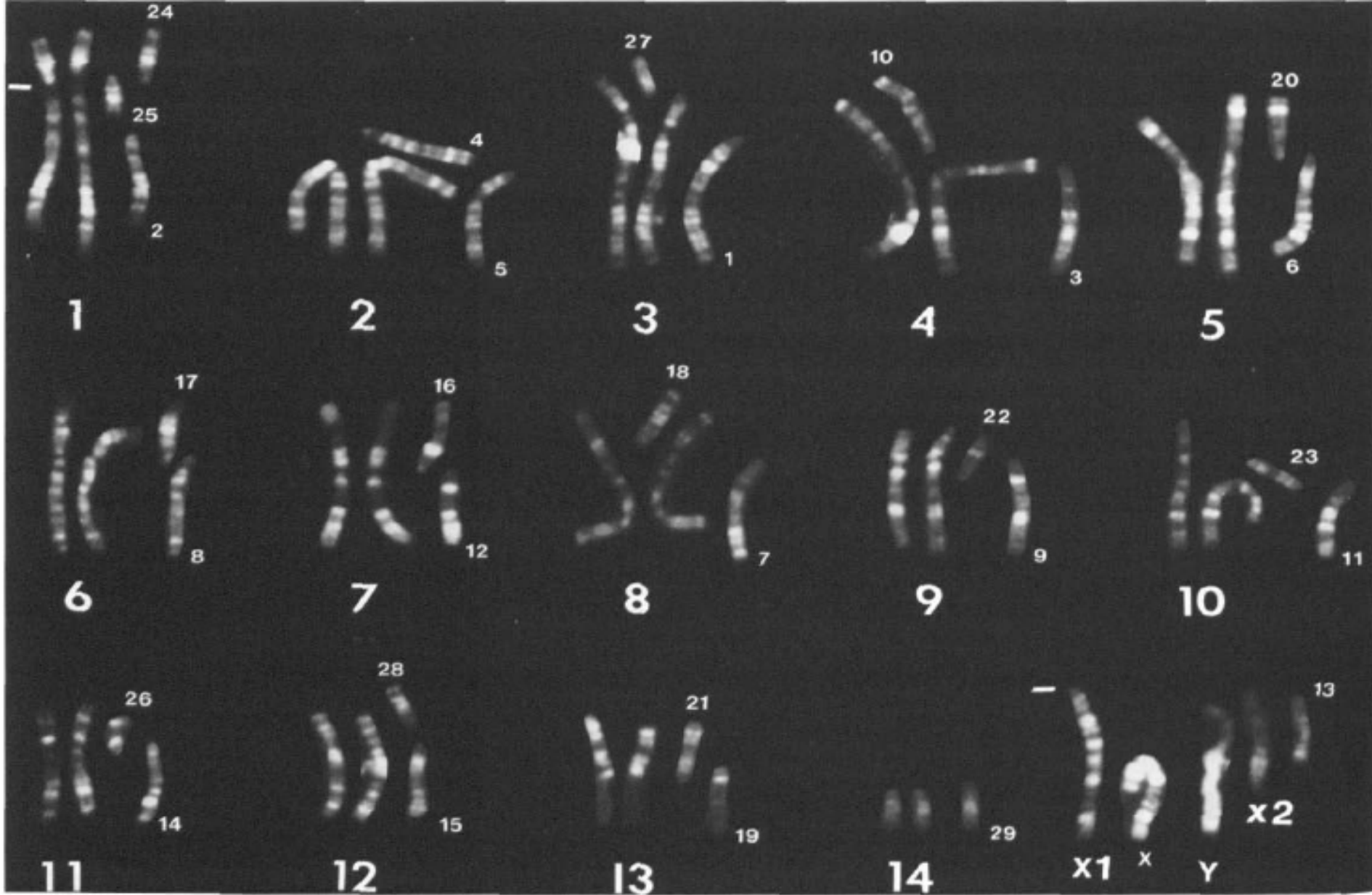
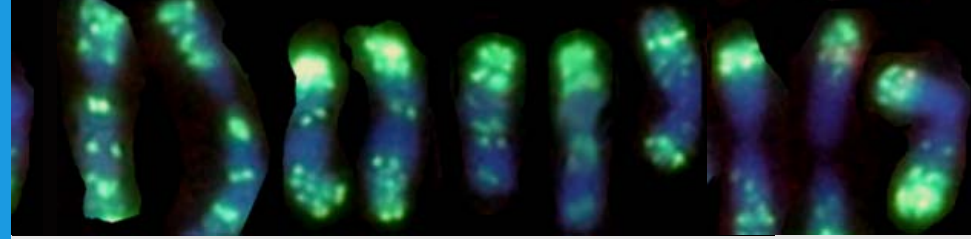
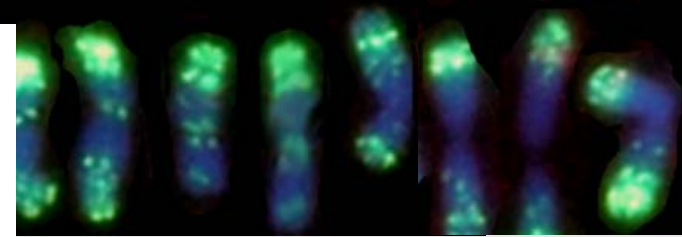


Figure 4. A male *Tragelaphus strepsicerus* (greater kudu) QFH-band karyotype ($2n = 31$) consisting of 26 banded autosomes, three acrocentric autosomes (one labeled as X2), an acrocentric X1, and a banded Y (the ancestral Y is fused to cattle equivalent autosome 13). The autosomal pairs are arranged and numbered (large numbers) according to relative size. The domestic cow equivalent chromosomes are arranged to the right of the greater kudu autosomes and are numbered (small numbers are placed toward the telomeric ends of the domestic cow acrocentric autosomes) according to the Reading Conference (1980) standard. The banding pattern of cattle chromosome 25 does not precisely match the region of kudu chromosome 1 to which we believe it is homologous, but this placement is the only way we found to account for cattle 25 within the kudu karyotype. The greater kudu sex chromosomes and equivalent cattle chromosomes are arranged from left to right as X1 (greater kudu ancestral X), cattle X, greater kudu Y, greater kudu X2, and cattle autosome 13. White lines are positioned at the centromere of some chromosomes.

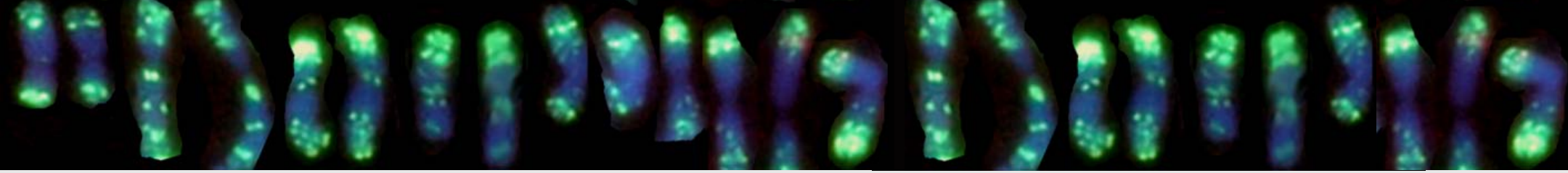


☛ Sheep *Ovis aries*

$2n=54, XY$
three pairs
biarmed
chromosomes
60 autosomal
arms



- Goat
- Sheep
- Cattle
- Chromosome homologies and centromeric fusions
- Paul Popescu



Bovidae Karyotype Evolution

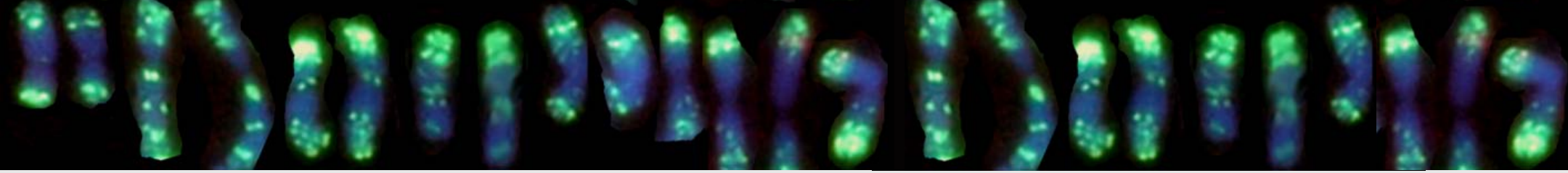
- ☞ $2n$ ranges from 30 to 60 in different species
- ☞ BUT:
- ☞ Almost all have 58 autosomal chromosome arms and two sex chromosomes
- ☞ (Galagher & Womak 1992)



Bovidae Karyotype Evolution

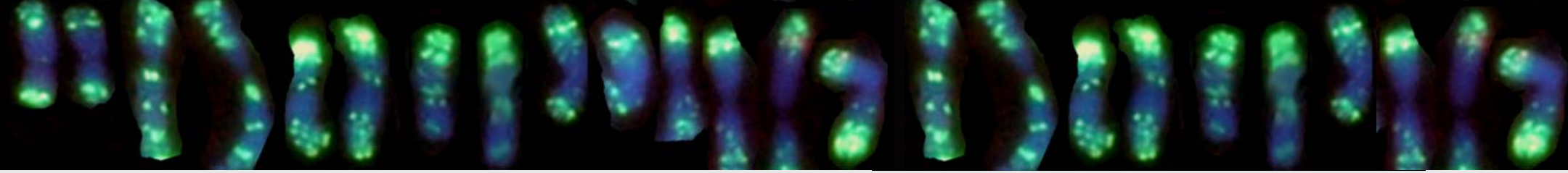
- Almost all have 58 autosomal chromosome arms and two sex chromosomes but $2n$ from 30 to 60
- Chromosome arm homologies extensive BUT homologous biarmed chromosomes are rare
- Reproductive isolation (and speciation) may have followed centric fusion

- (Galagher & Womak 1992)



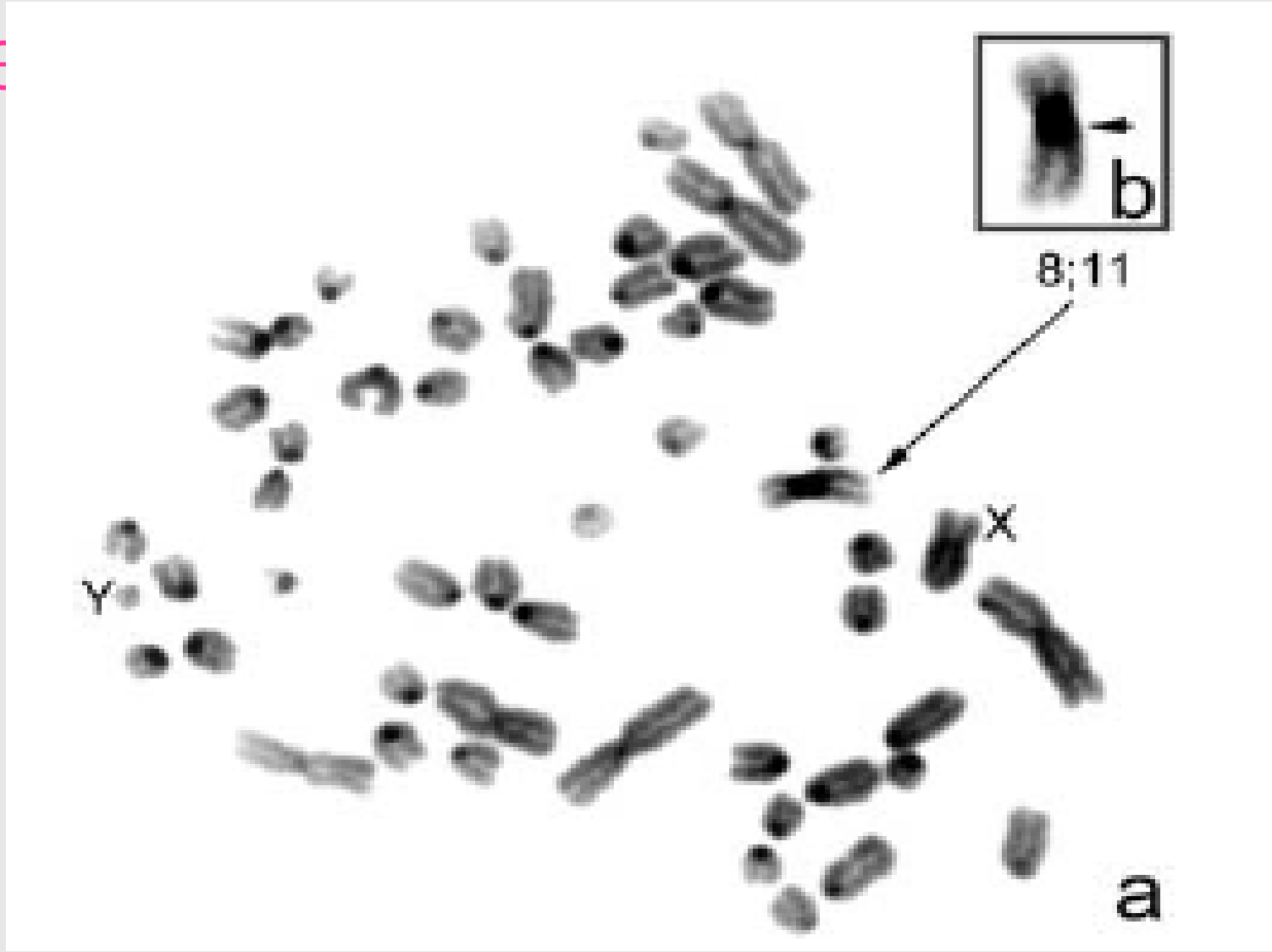
Do we see chromosome fusion now?

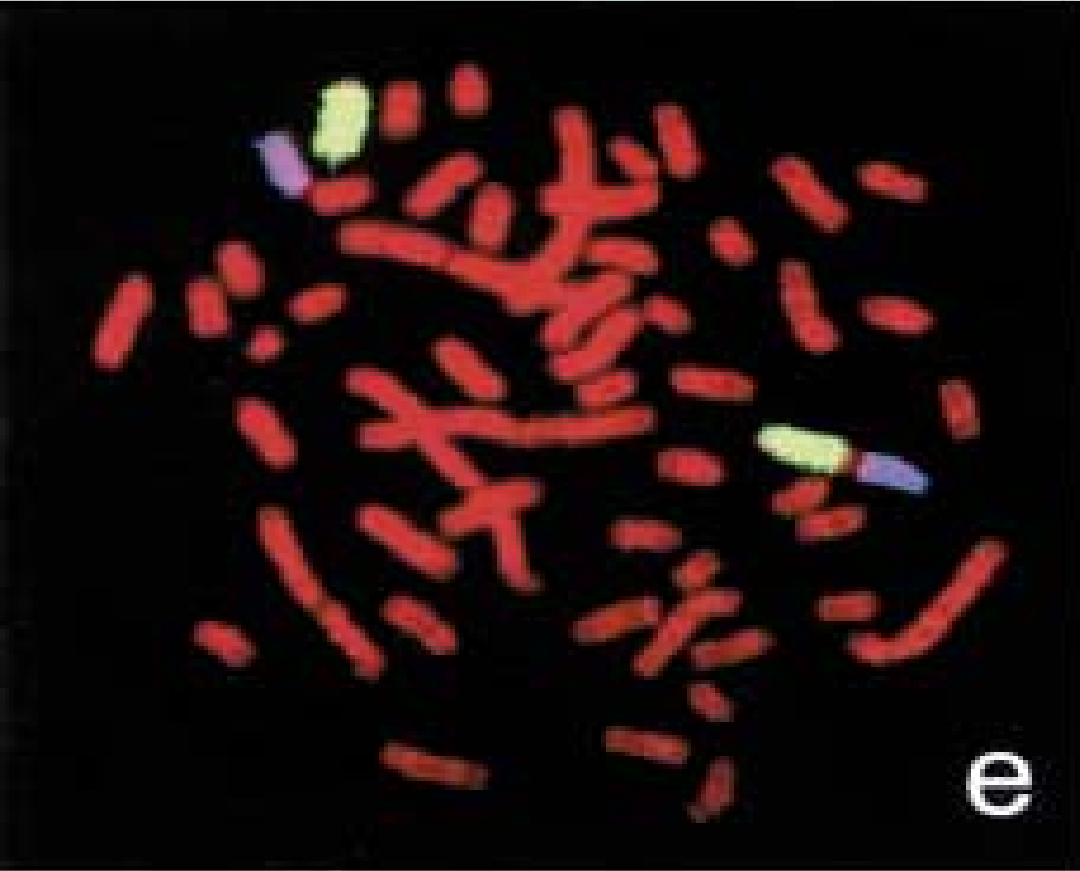
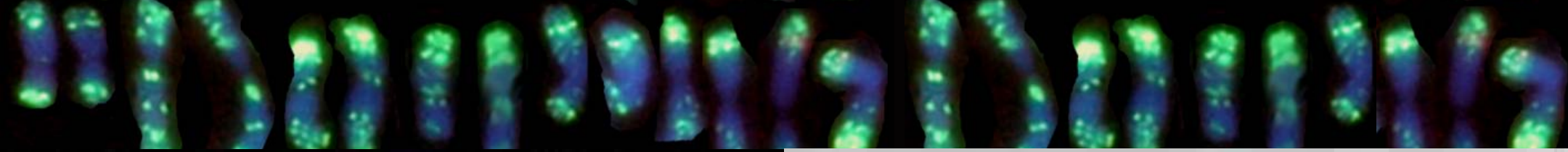


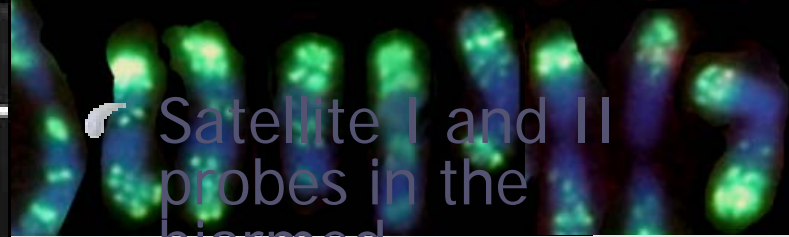
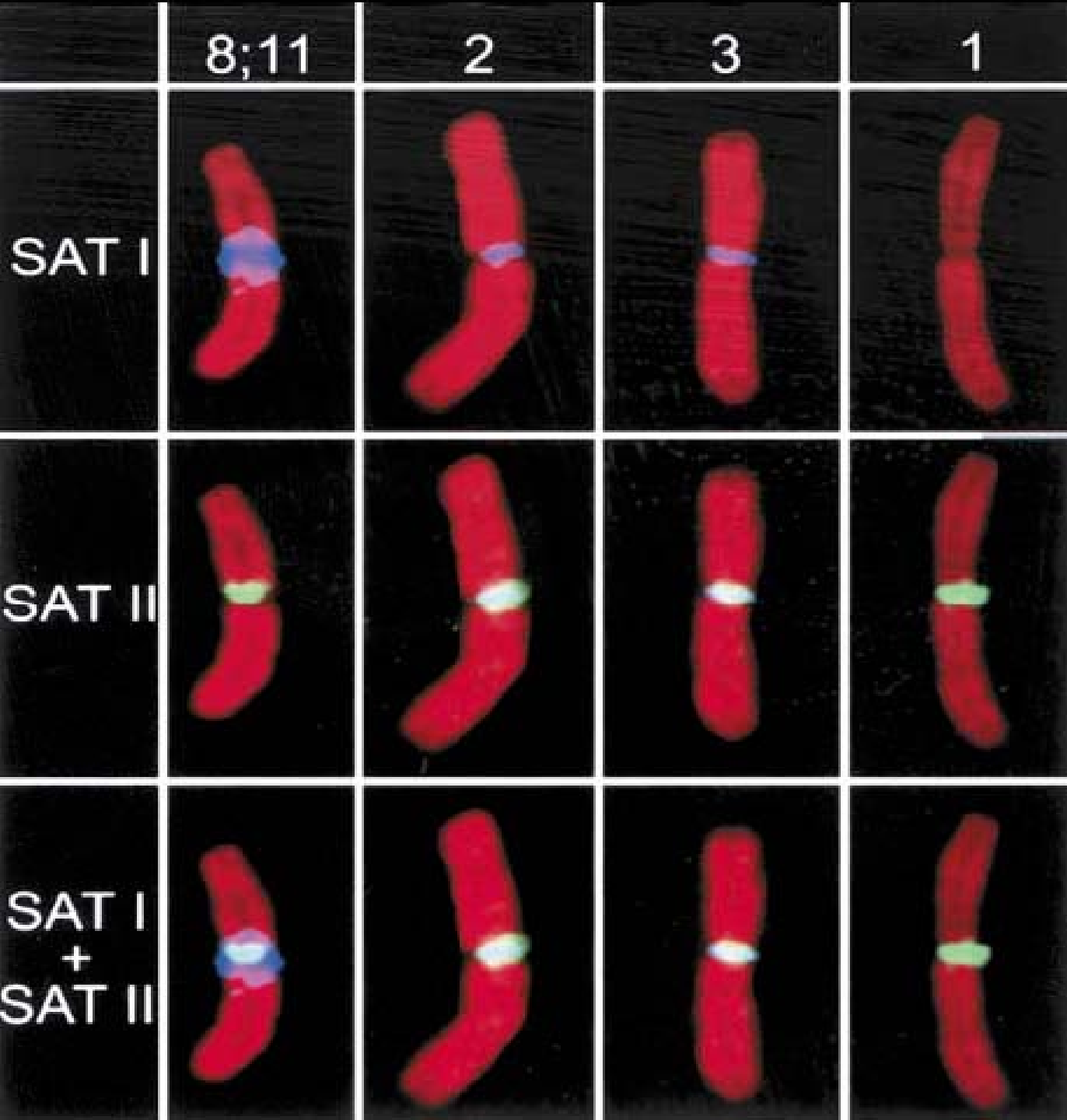


Do we see

Molecular cytogenetic analysis and centromeric satellite organization of a novel 8;11 translocation in sheep: a possible intermediate in biarmed chromosome evolution. 2003. Chaves, Adegas, Wienberg, Guedes-Pinto, Heslop-Harrison

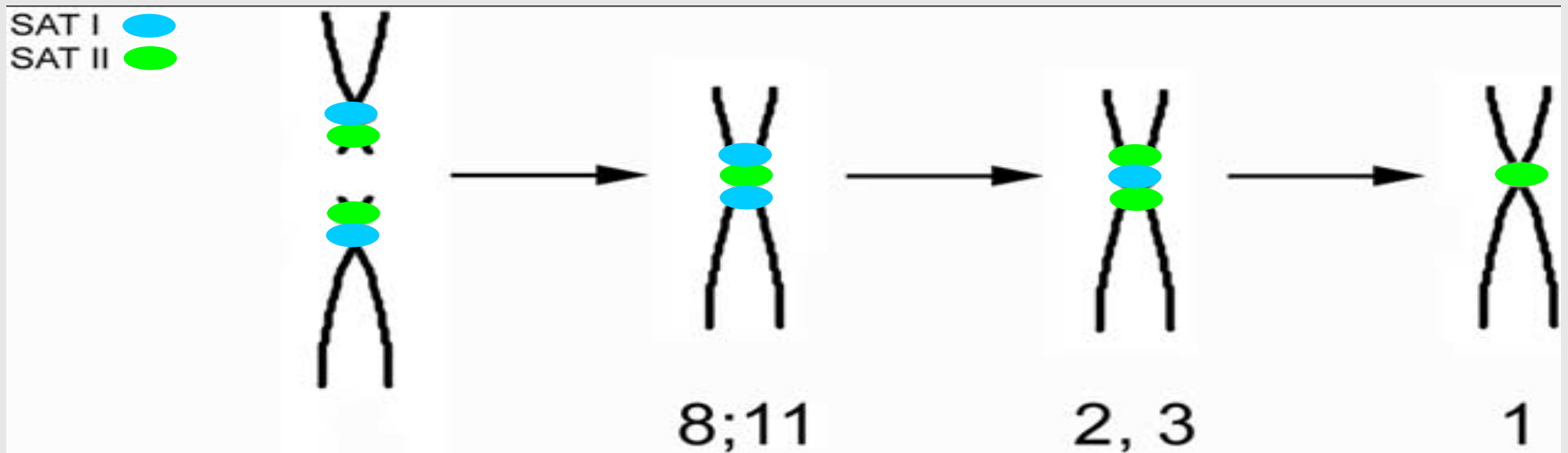






- Satellite I and II probes in the banded chromosomes of the sheep with $2n = 53, XY$.
- Chr (8;11), 2, 3, 1 are ordered from the most recent to the postulated evolutionarily oldest chromosome

t(8;11) showed satellite I proximal on both arms with satellite II covering the centromere, while the evolutionarily derived fusion leading to Chrs 2 and 3 showed the opposite configuration, not obviously derived by a simple fusion. Chr 1 has lost the satellite I hybridization patterns. The novel t(8;11) provides strong evidence for an intermediate step in evolution of the biarmed chromosomes in sheep.



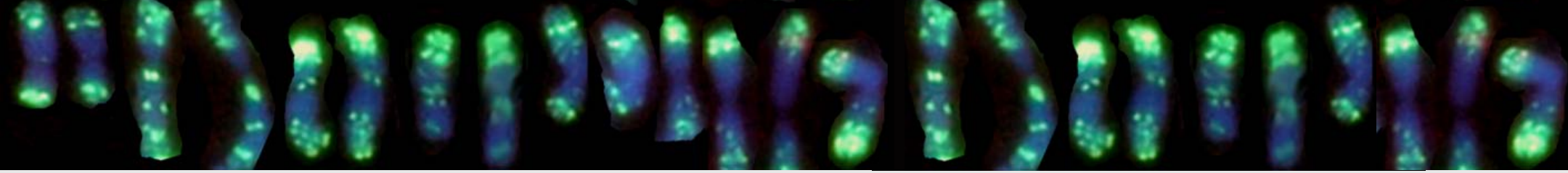
How many chromosomes?

☞ Is the number constant in a species?

☞ Cattle $2n=60$

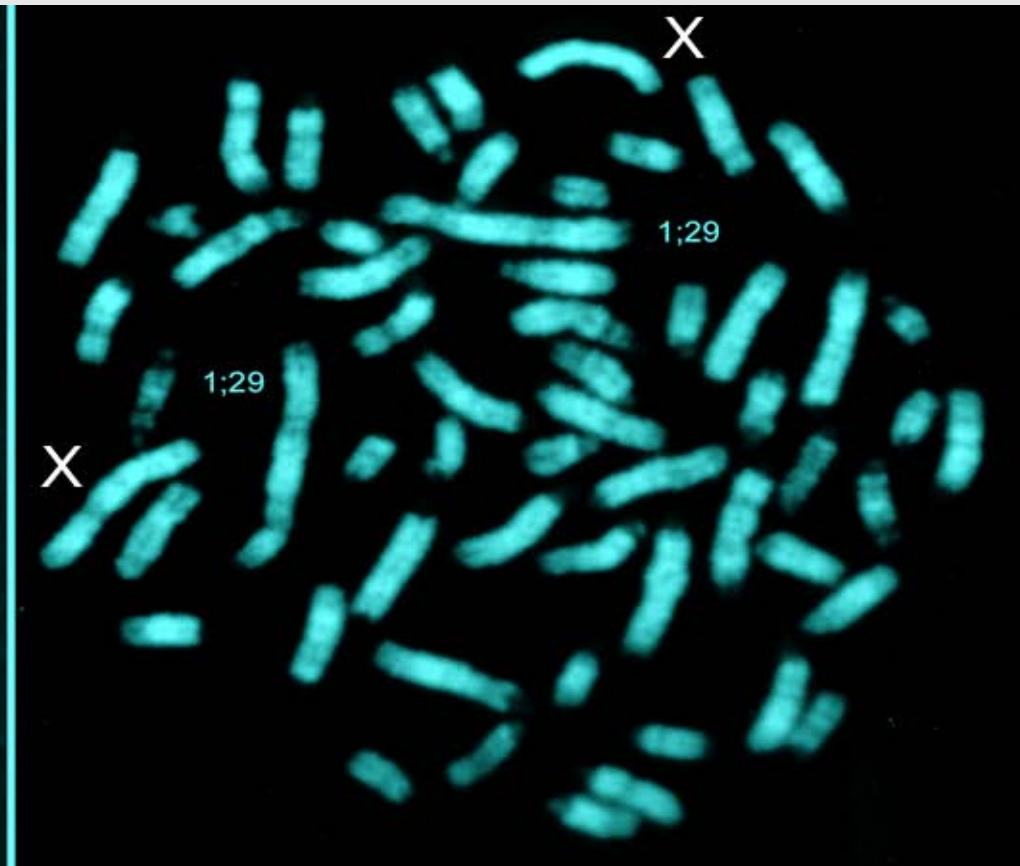
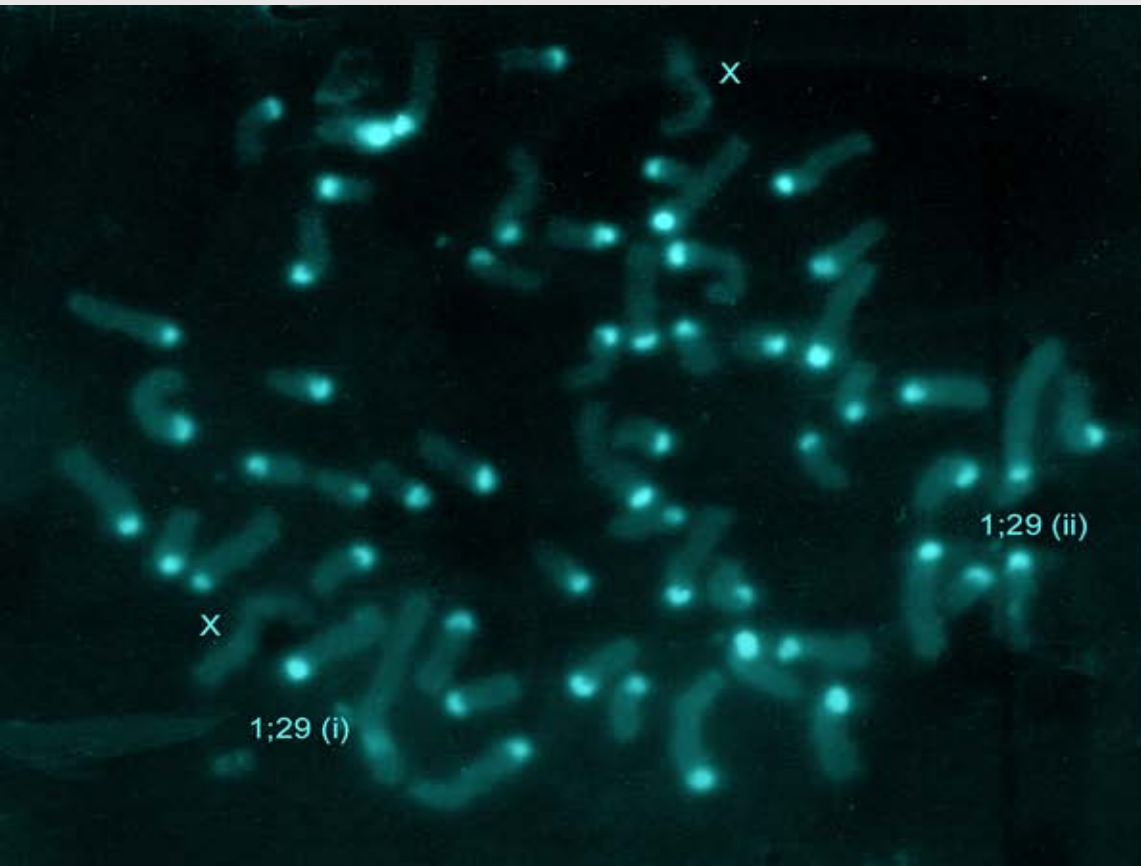
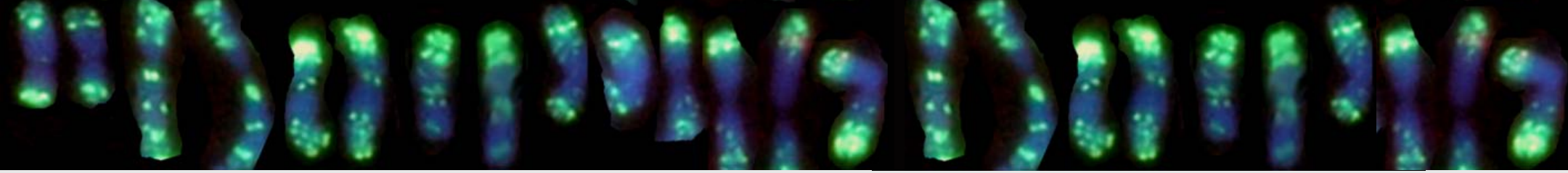
● but some individuals have $2n=58$
or $2n=59$ because two
chromosomes fuse

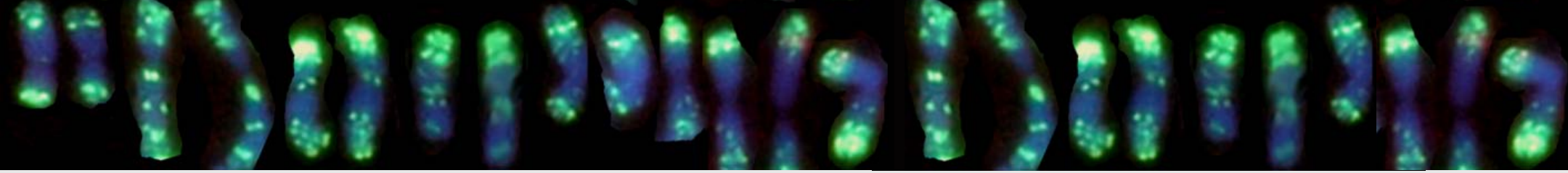
☞ Chromosomal evolution is happening now



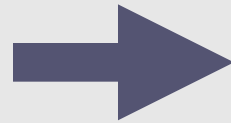
The 1;29 fusion in cattle

- Found in multiple breeds
- Sometimes a founder effect (imported in one bull – e.g. Brahman to Africa)
- But present even in major breeds
- Limited effect on fertility
- Probably positively selected for a difficult-to-score trait

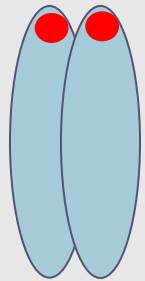




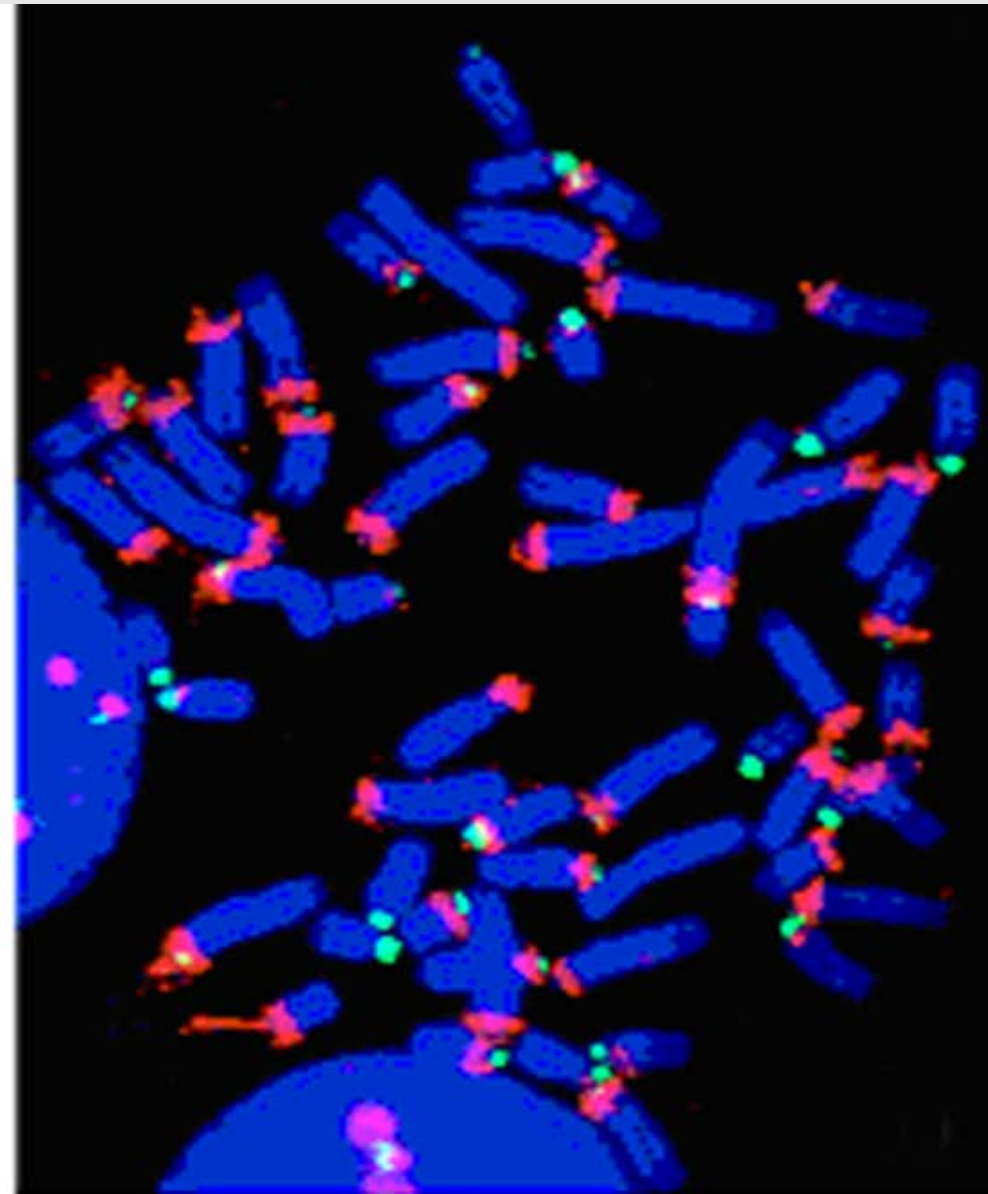
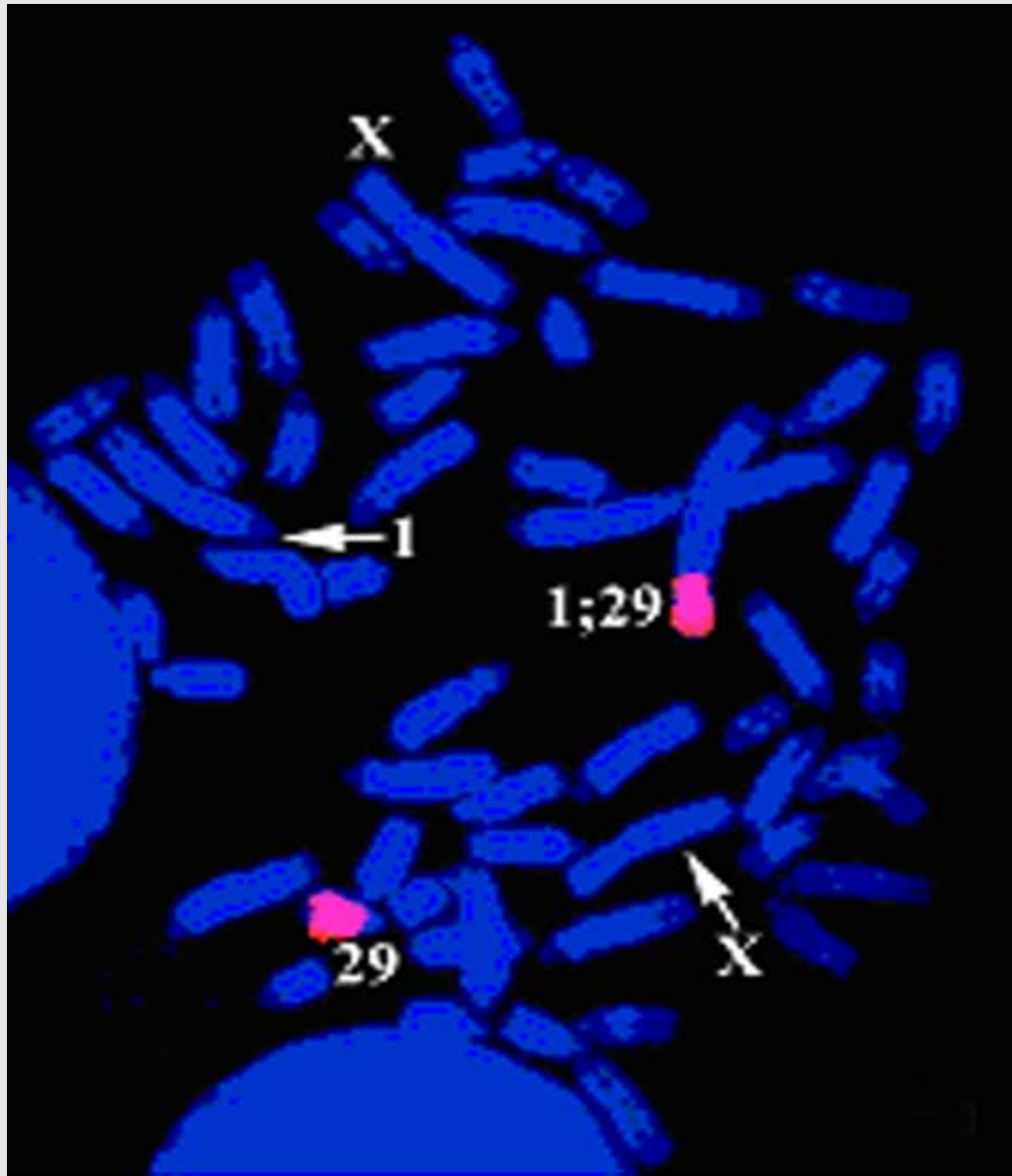
Robertsonian Fusion

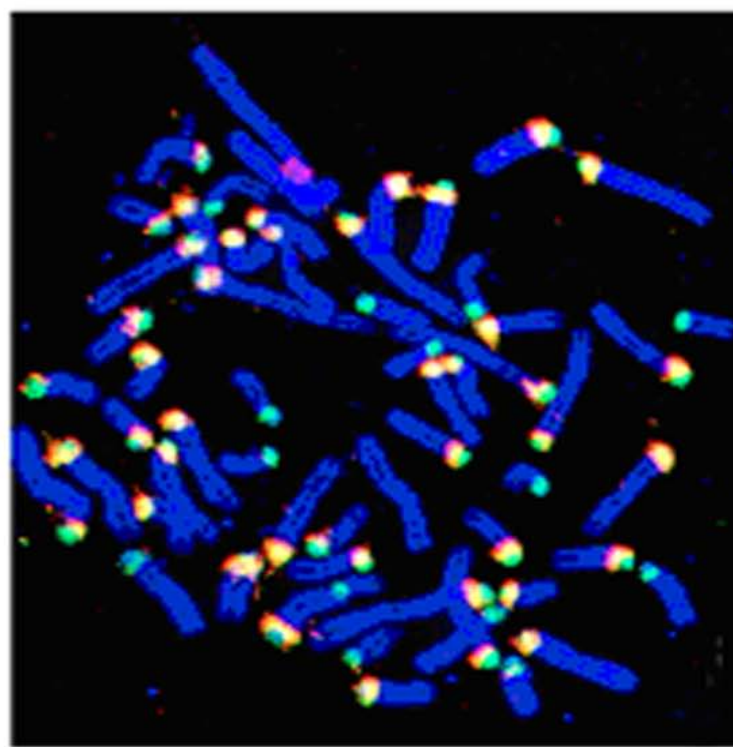
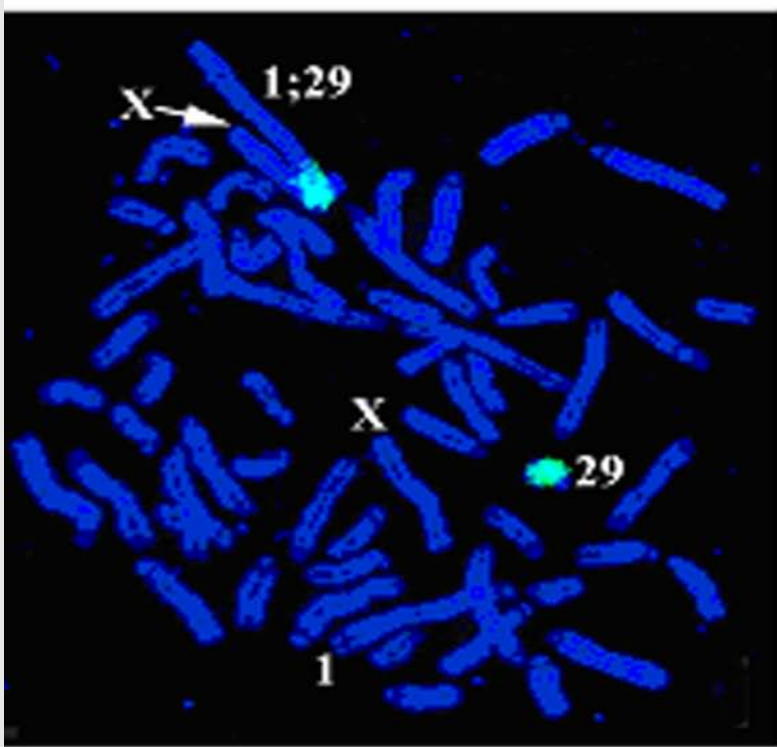
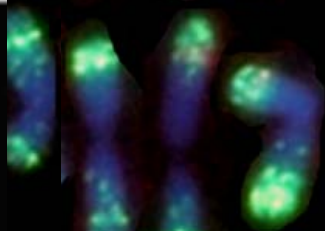
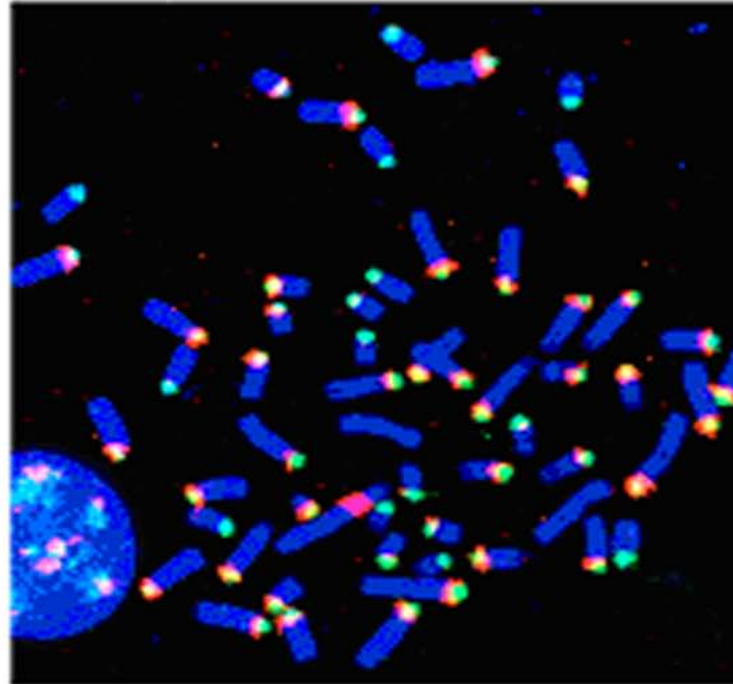
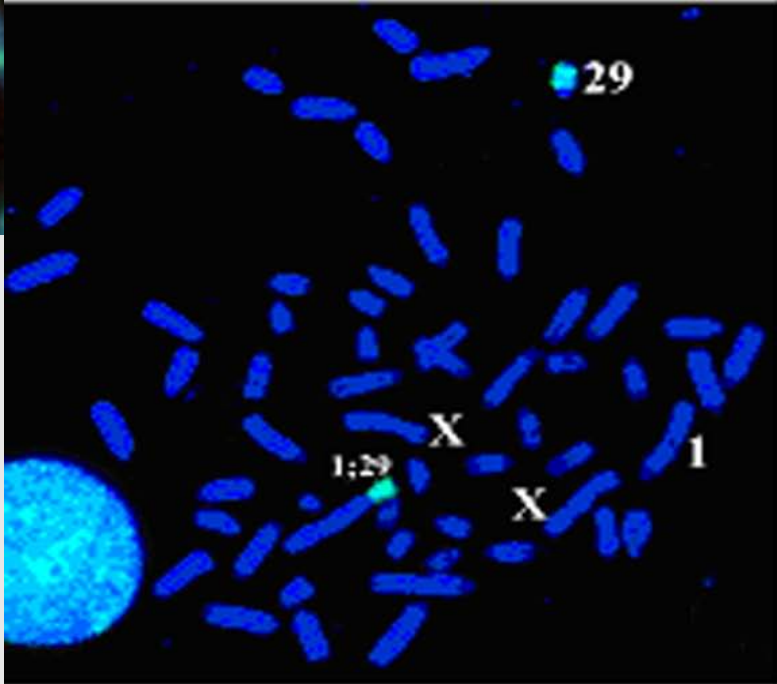
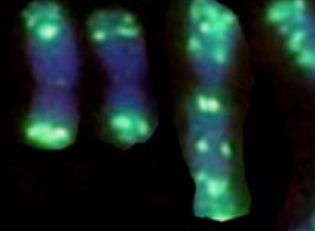


(+ ●● ?)



Bovine alpha-satellites and chromosome evolution

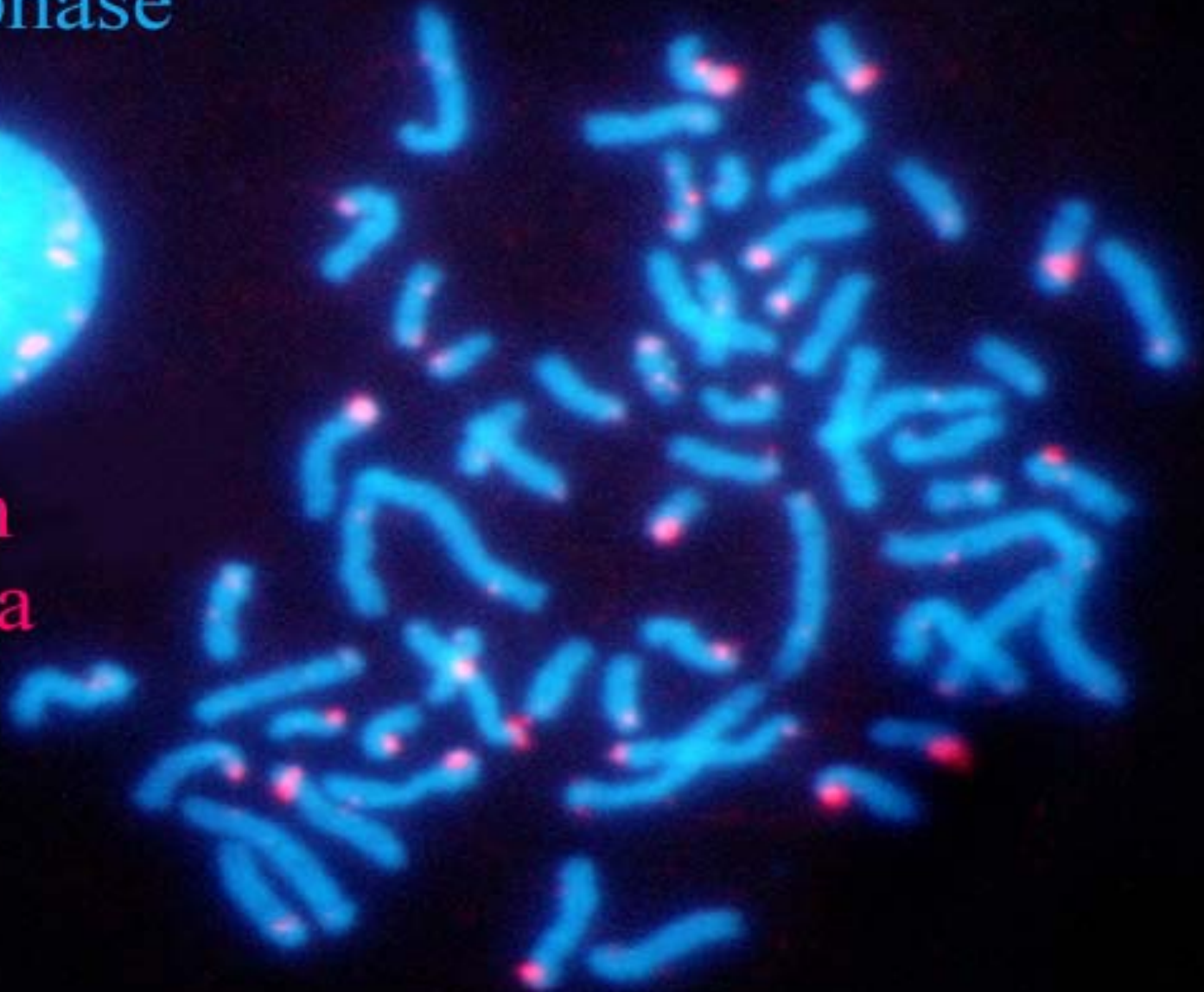




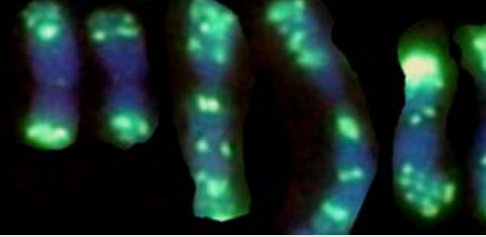
Goat Metaphase



Probed with
Sheep Alpha
Satellite



Raquel Chaves
& HH 1998



29

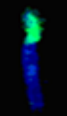
SAT IV



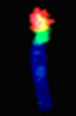
SAT IV



SAT I



SAT IV



SAT I



SAT I

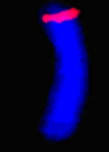


1

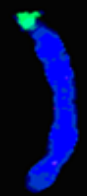
SAT III_{pvu}



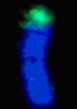
SAT III_{sau}



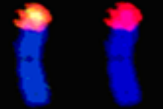
SAT I



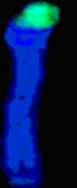
SAT I



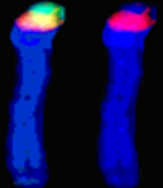
SAT III_{pvu}



SAT I

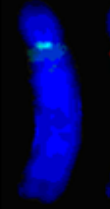


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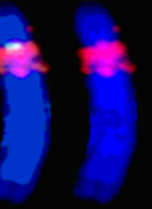


1;29

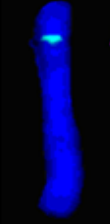
SAT IV



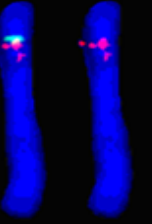
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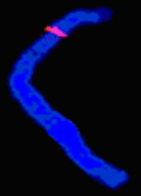
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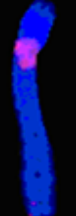
SAT IV



SAT III_{pvu}



SAT III_{sau}

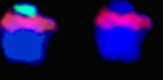


remaining acrocentrics

SAT IV



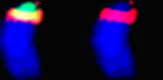
SAT III_{pvu}



SAT IV



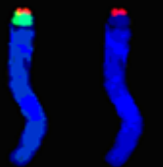
SAT III_{sau}



SAT I



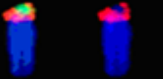
SAT IV



SAT I



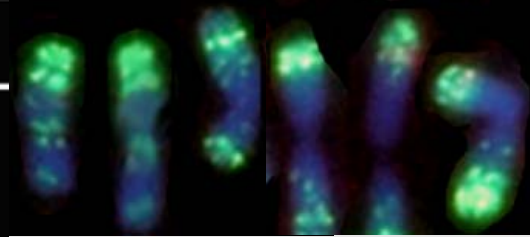
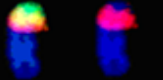
SAT III_{pvu}



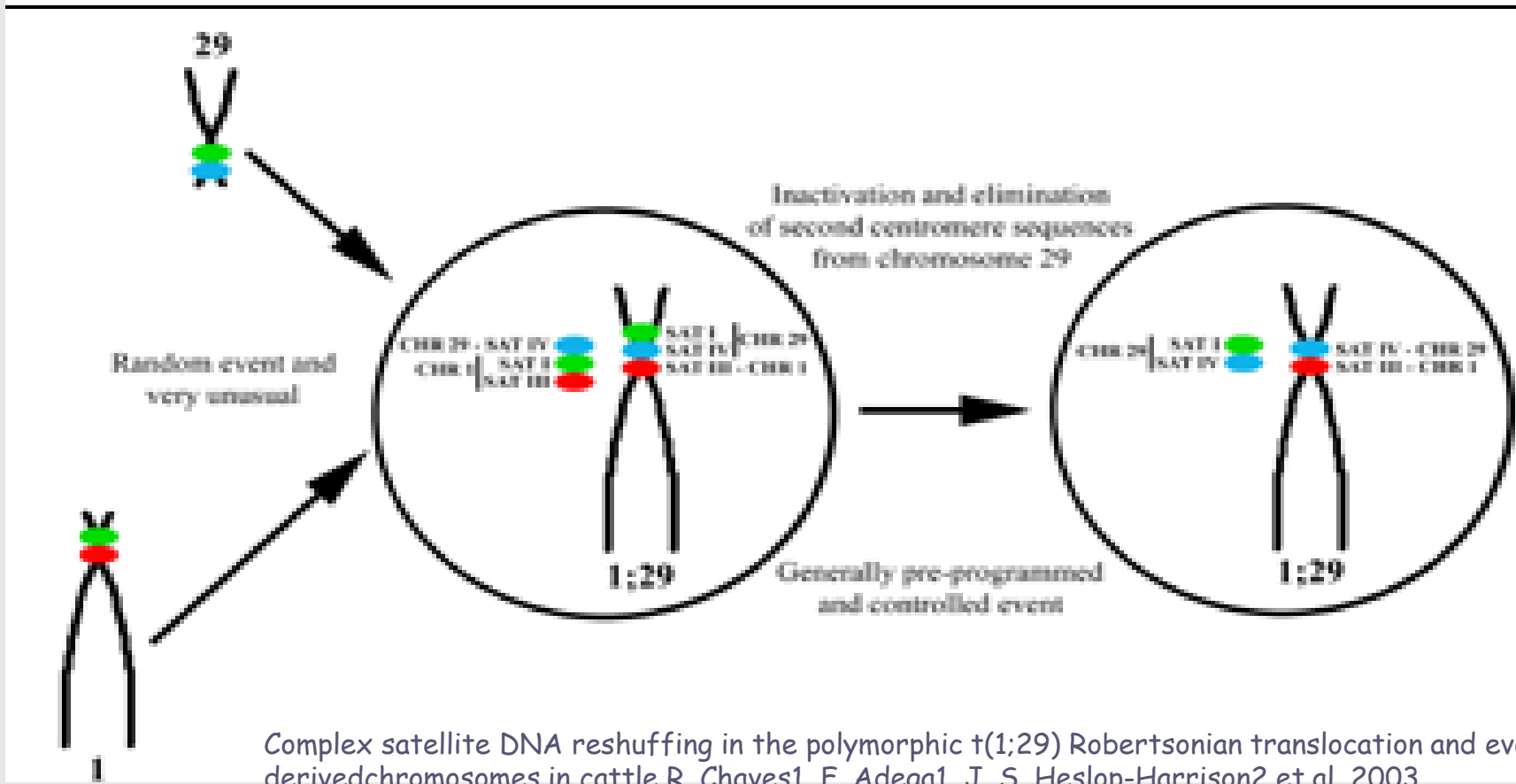
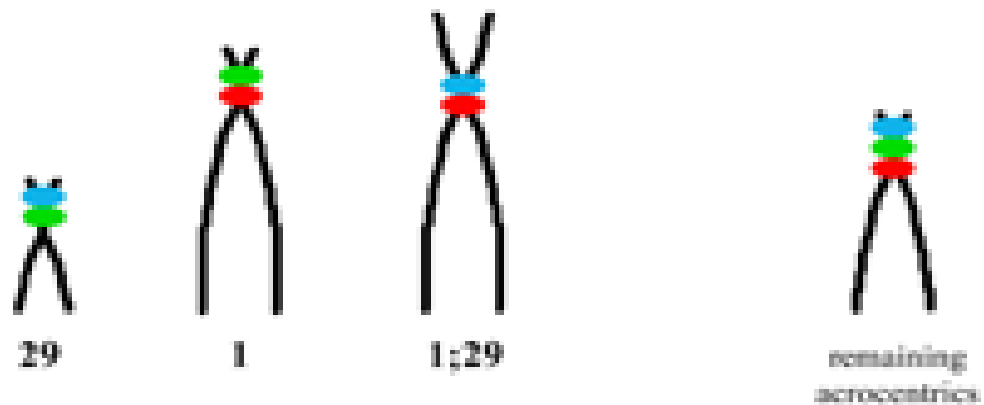
SAT I



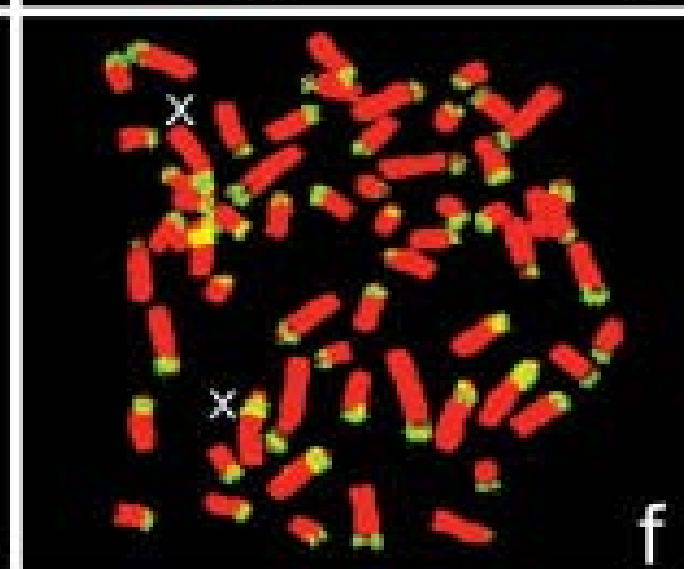
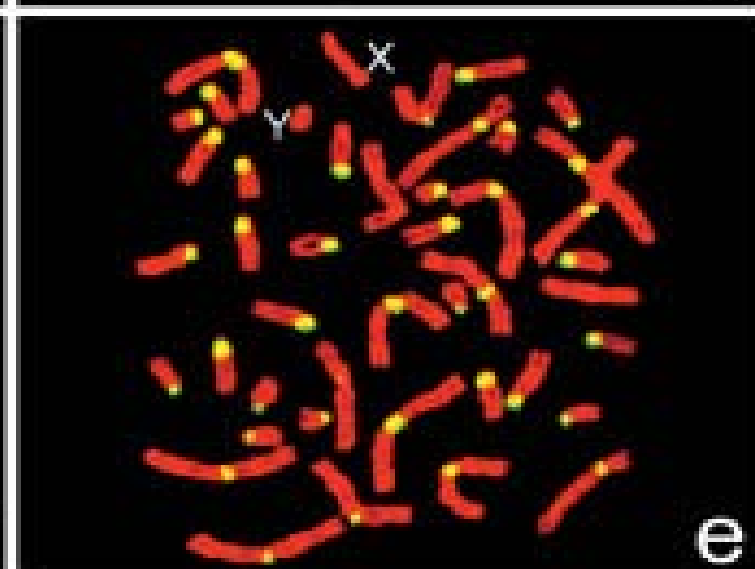
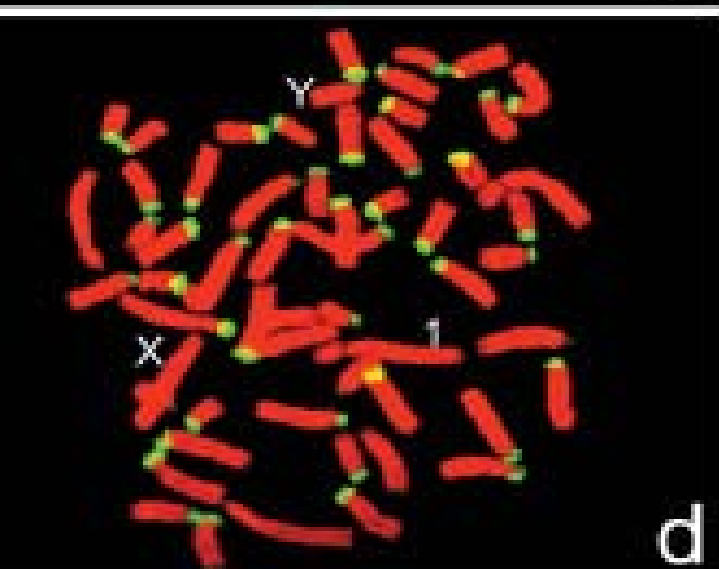
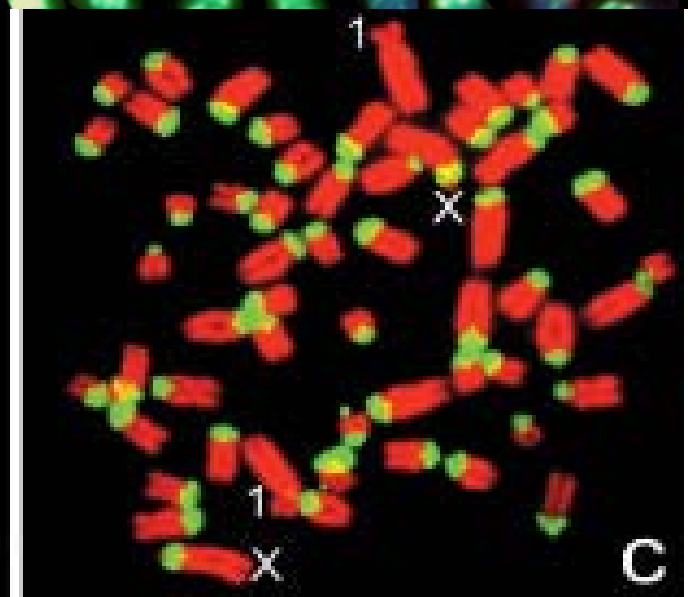
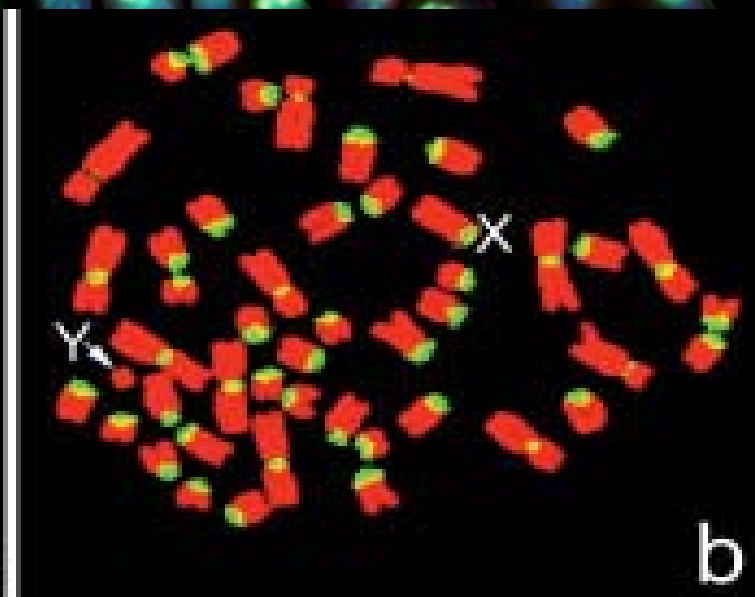
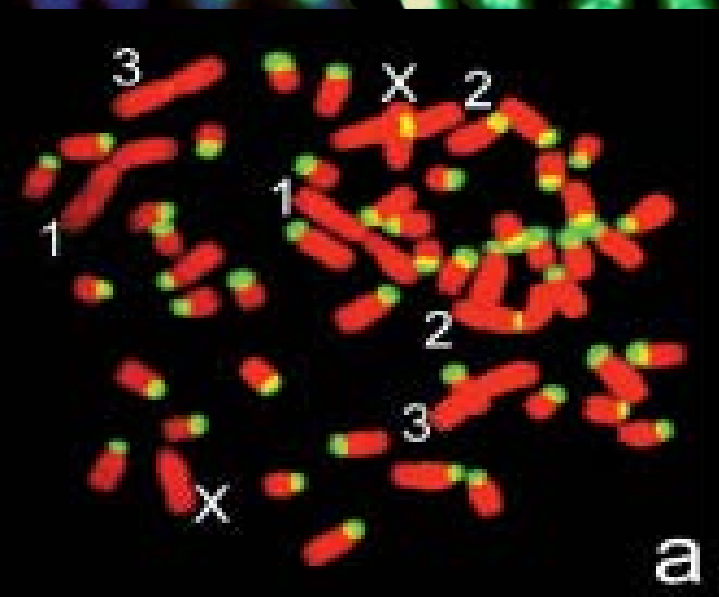
SAT III_{sau}

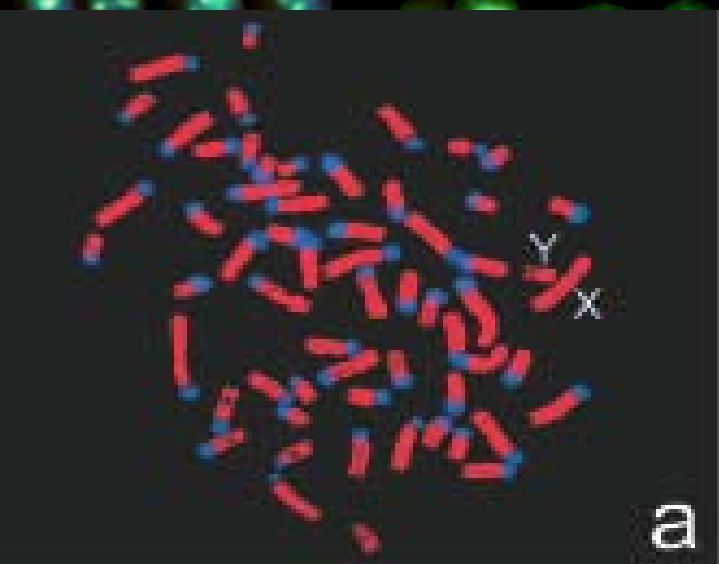


SAT I (blue)
 SAT III (red)
 SAT IV (green)

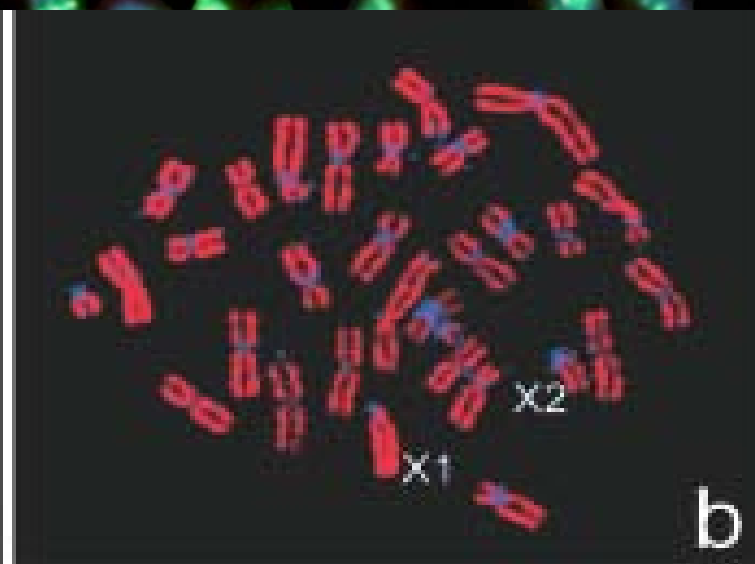


Complex satellite DNA reshuffling in the polymorphic t(1;29) Robertsonian translocation and evolutionarily derived chromosomes in cattle R. Chaves¹, F. Adegas¹, J. S. Heslop-Harrison², et al. 2003

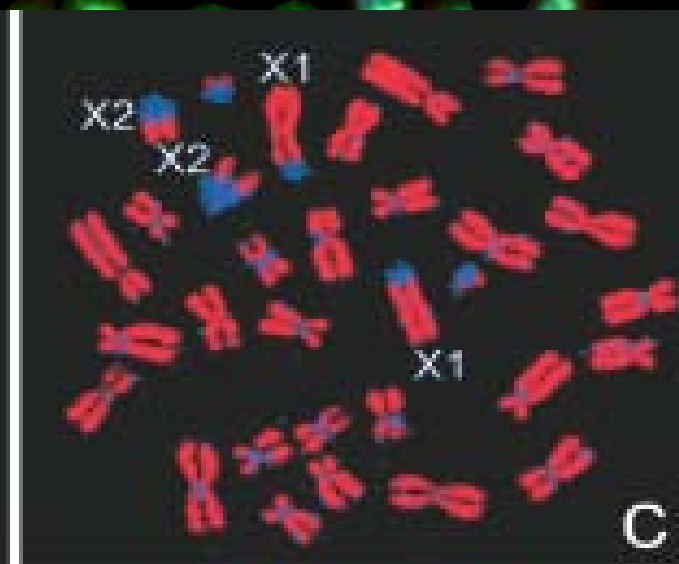




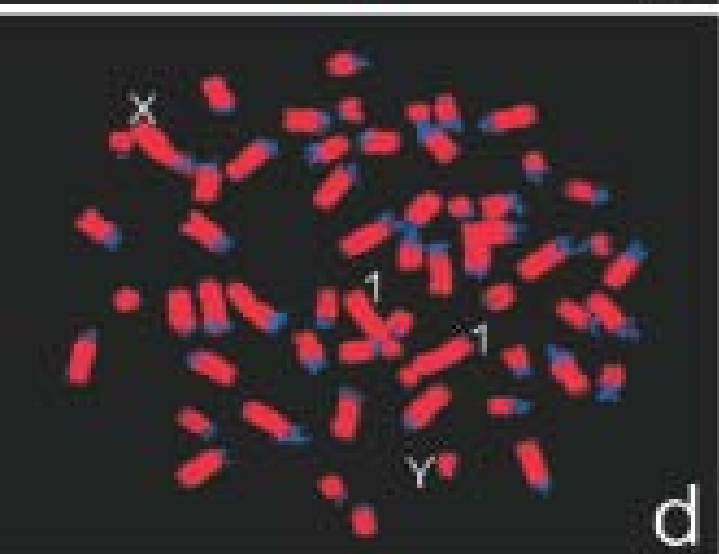
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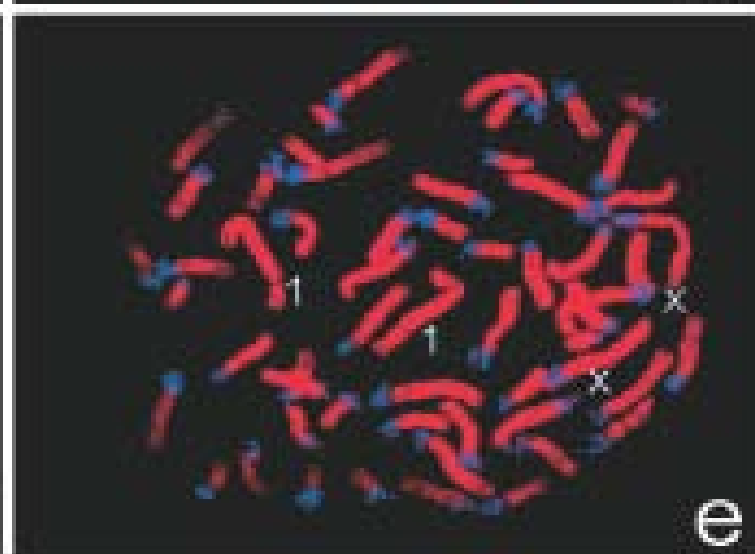
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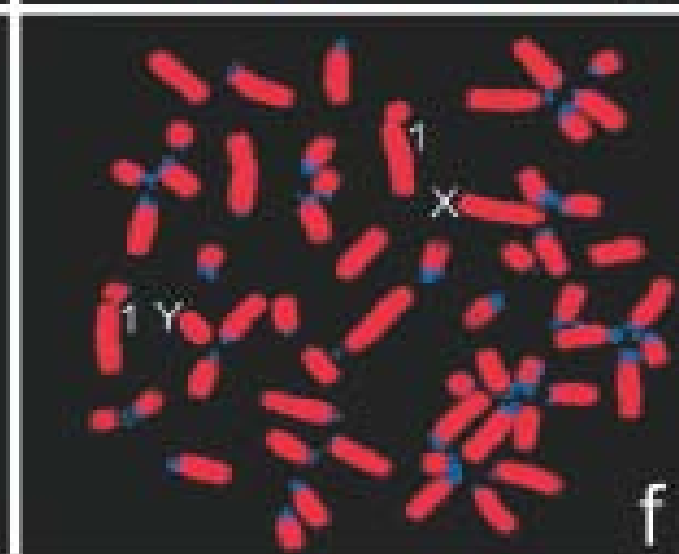
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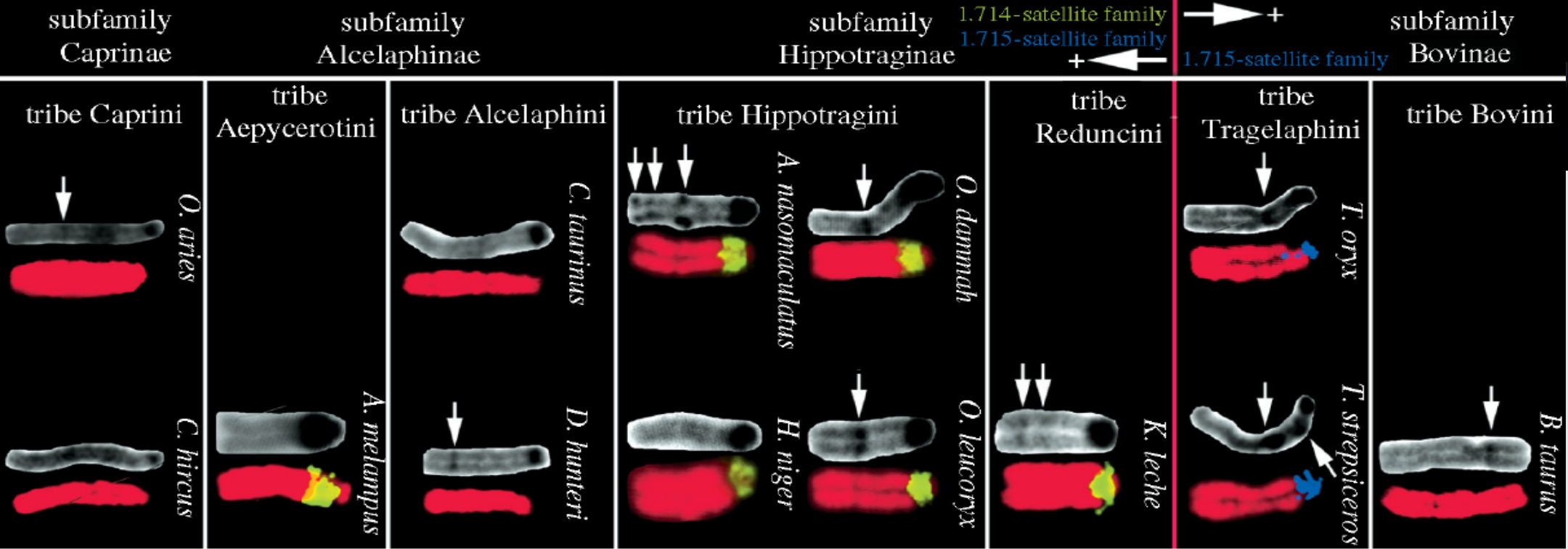
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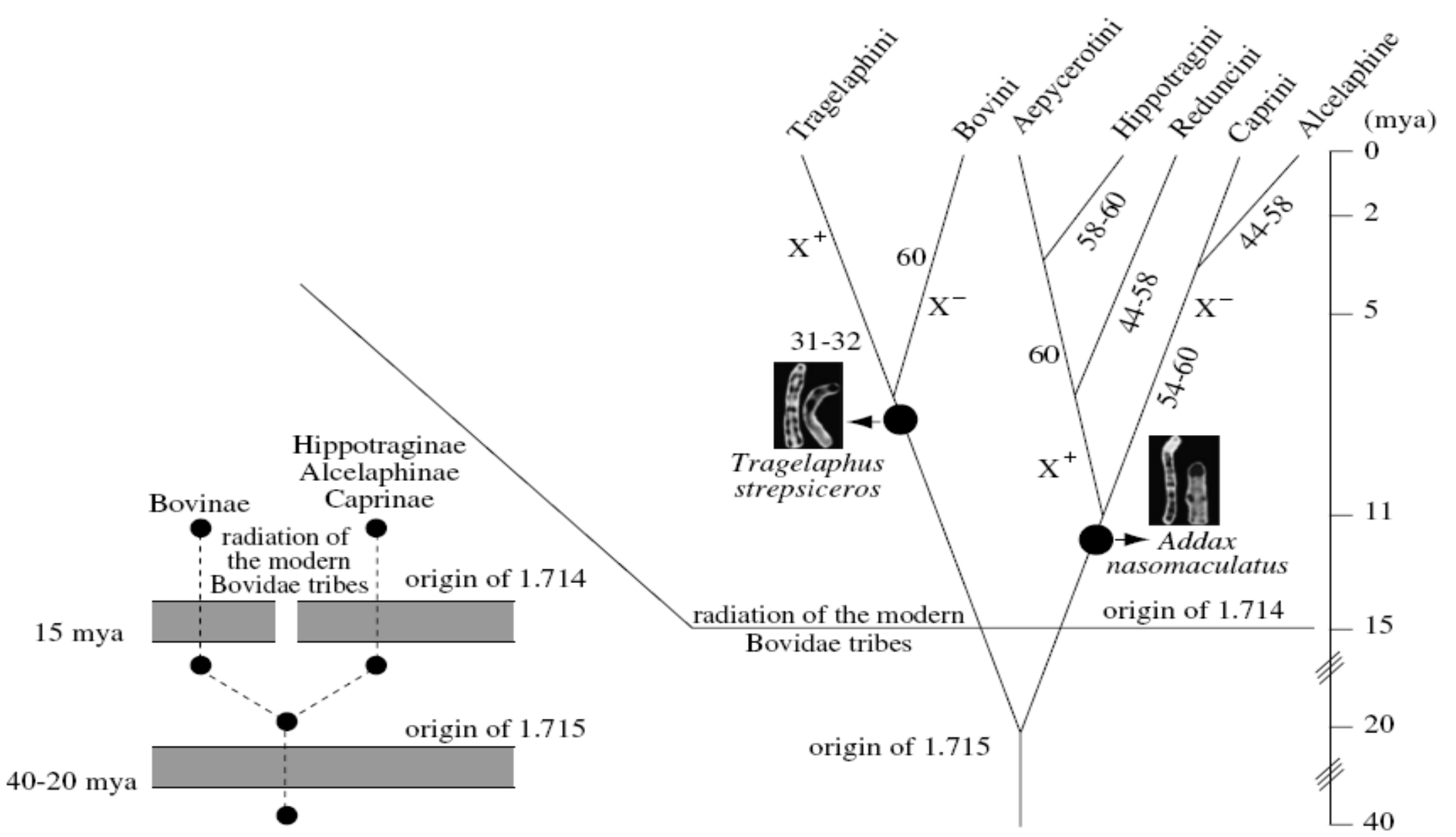
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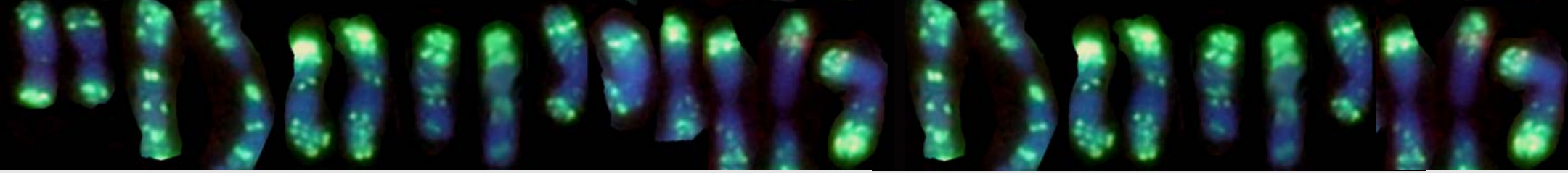
f



Hybridization of sheep (clone pOaKB9) and cattle (pBtKB5) DNA satellite I probes to the X chromosome of most representative species listed in table 1. Bovinae subfamily shows only hybridization with the cattle satellite I and only in the X chromosome's centromeric regions of the tribe Tragelaphini was there signal from the cattle satellite I. Metaphases of subfamilies Hippotraginae, Alcelaphinae and Caprinae show positive in situ hybridization signals with both sheep and cattle satellite I probes. However, only the X chromosome centromeric regions of the Tribes Reduncini, Hippotragini and Aepycerotini show positive in situ hybridization signals with both satellite probes



Phylogenetic relationships and the primitive X chromosome inferred from chromosomal and satellite DNA analysis in Bovidae Raquel Chaves^{1,*}, Henrique Guedes-Pinto¹ and John S. Heslop-Harrison Proc Roy Soc B 2005



Bovidae – Family

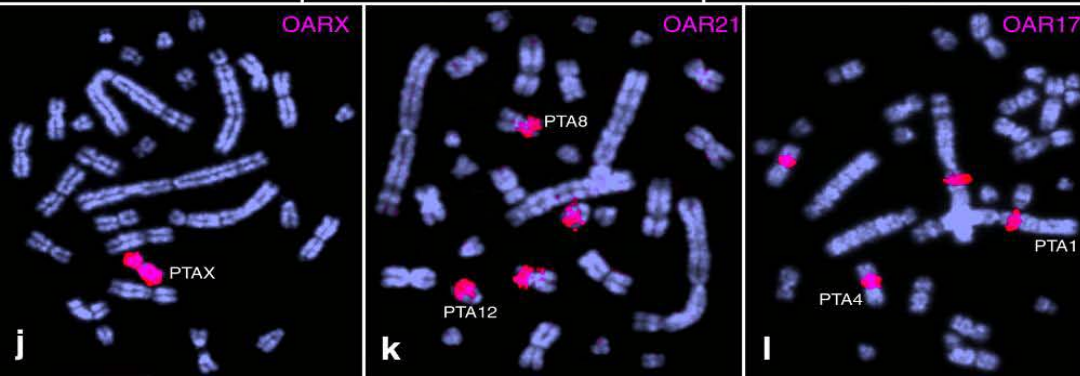
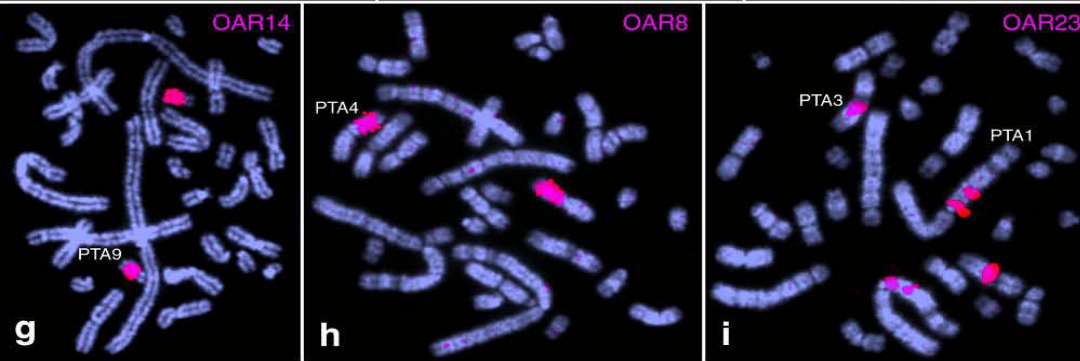
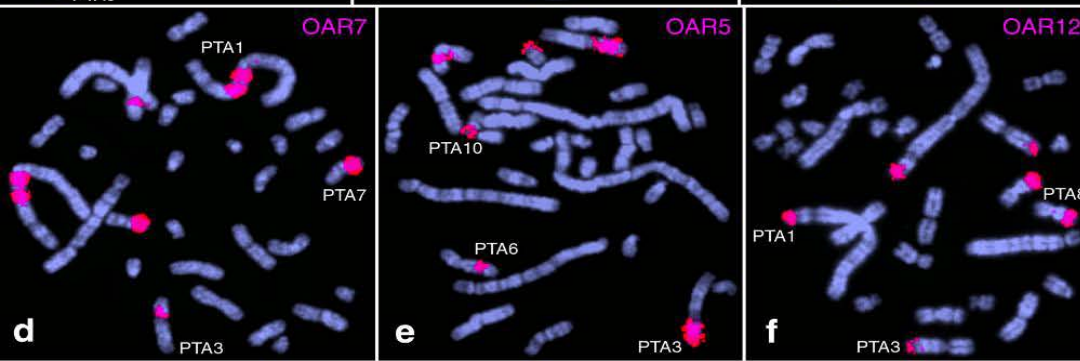
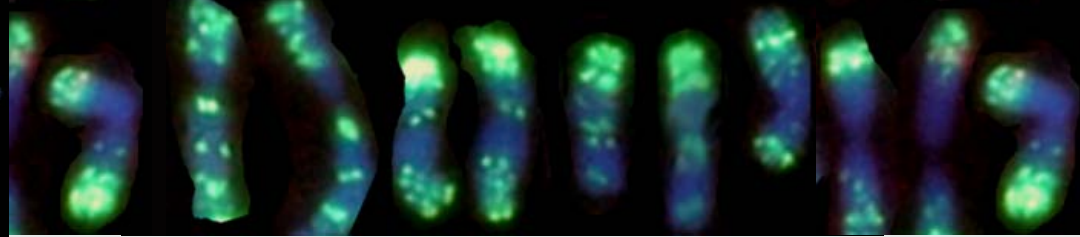
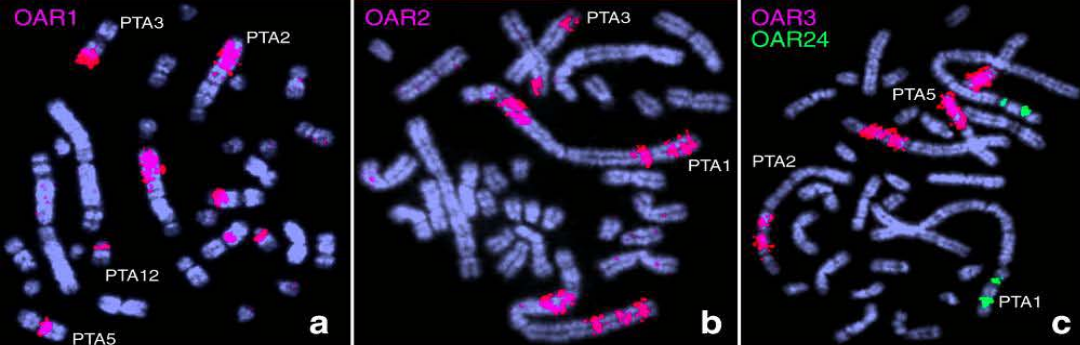
☞ Mammals

- Order Artiodactyla (=Even-toed ungulates)
- 3 groups: the Suiformes (pigs, peccaries, hippopotamuses), Tylopoda (camels, llamas) and Ruminantia (cattle, goats, sheep, deer, antelopes, giraffes)
- 9 families (13 tribes) including Bovidinae
 - Family Bovidae
 - c. 137 species
 - Last species (new genus) discovered in 1992

Translocation in Sus

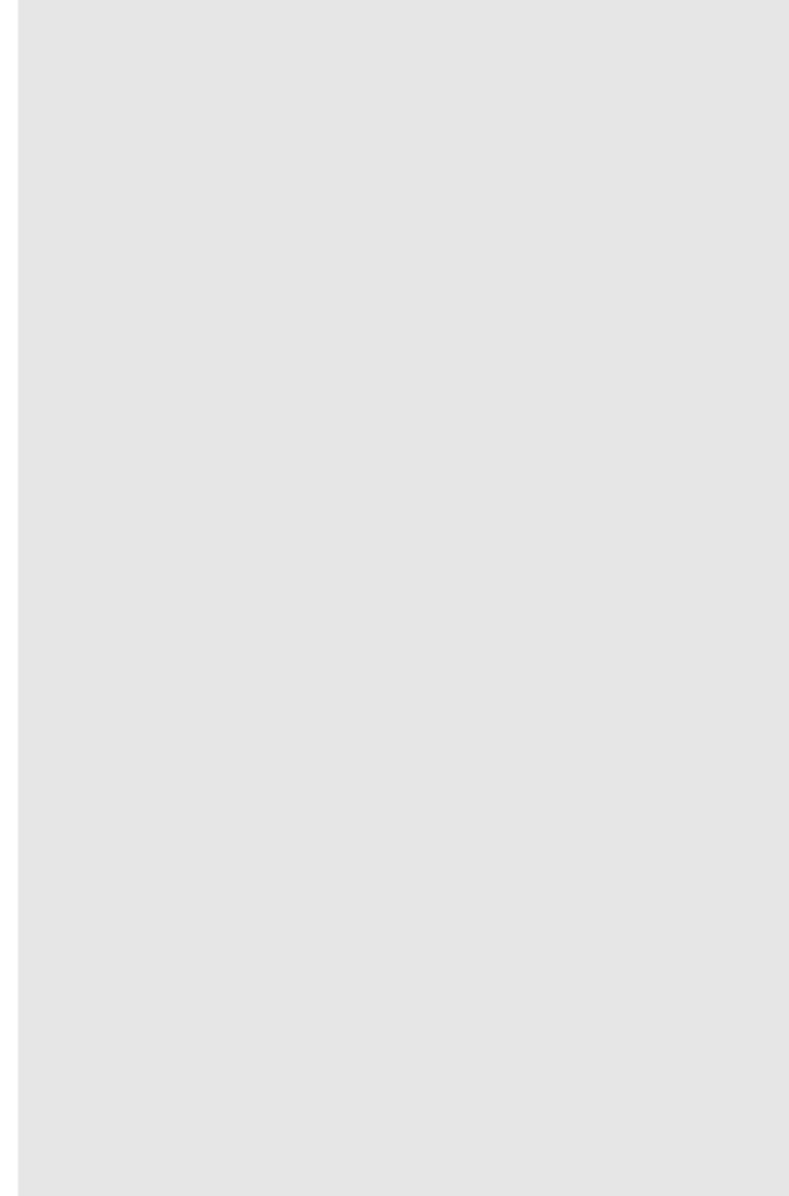
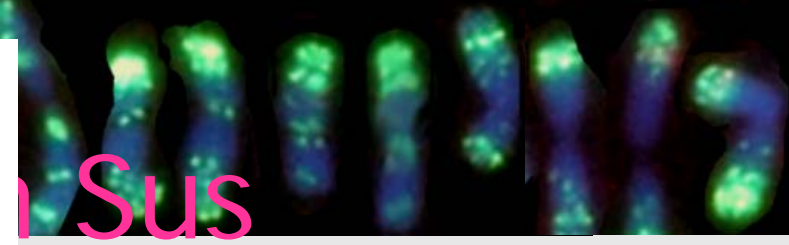
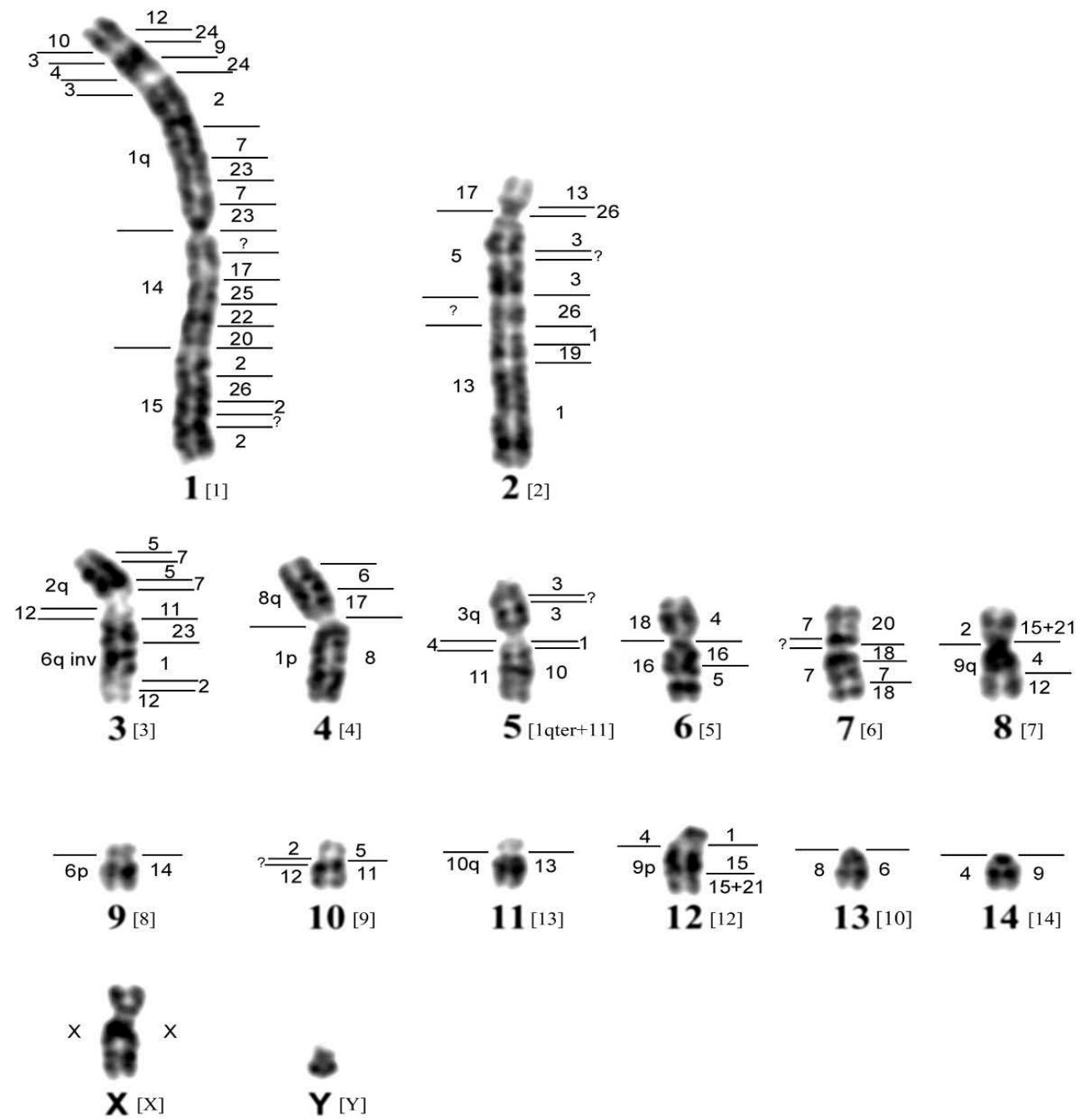


High-resolution comparative chromosome painting in the Arizona collared peccary (*Pecari tajacu*, *Tayassuidae*): a comparison with the karyotype of pig and sheep Filomena Adegá, Raquel Chaves, Andrea Kofler, Paul R. Krausman, Julio Masabanda, Johannes Wienberg & Henrique Guedes-Pinto. *Chromosome Research* 2006



Translocation in Sus

SSC OAR







Diversity

- In the genepool
 - Allelic in one species
 - In different genomes
 - In different species
- From recombination
- From new mutations
 - Induced or natural
 - DNA sequence or retroelements



Polyploidy

- **Polyploids** have three or more complete sets of chromosomes

☞ diploid $2n=2x$

☞ triploid $2n=3x$

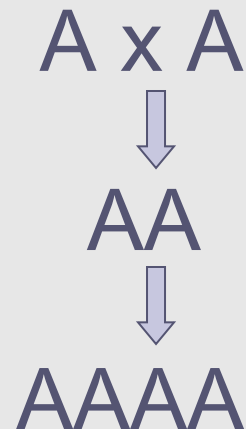
☞ hexaploid $2n=6x$

☞ tetraploid $2n=4x$

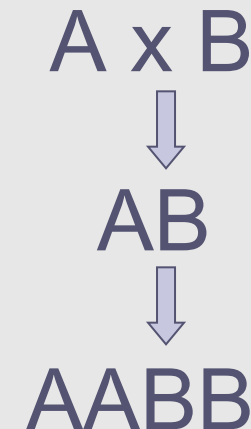
☞ octoploid $2n=8x$

- Two types of polyploidy

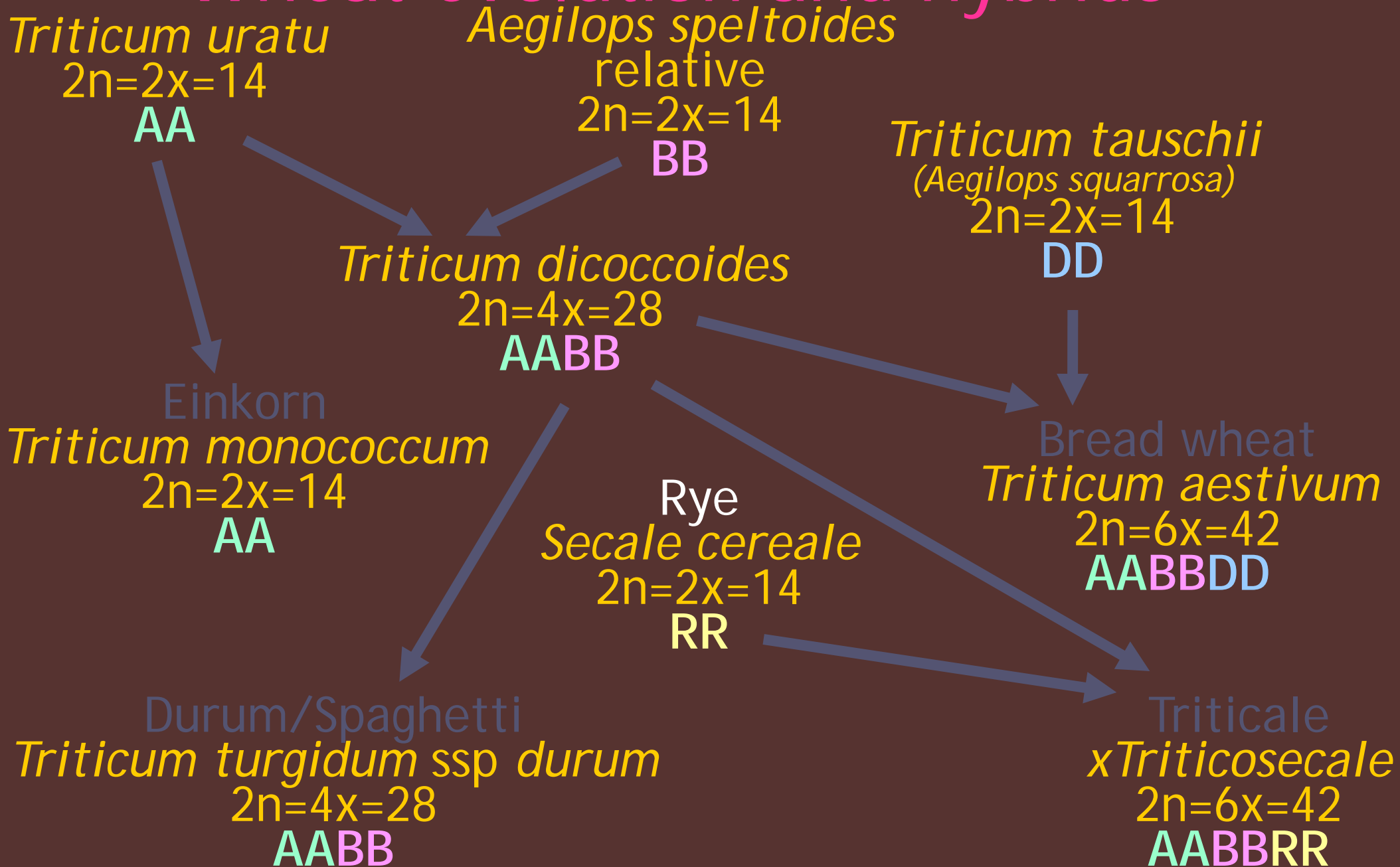
Autopolyploidy

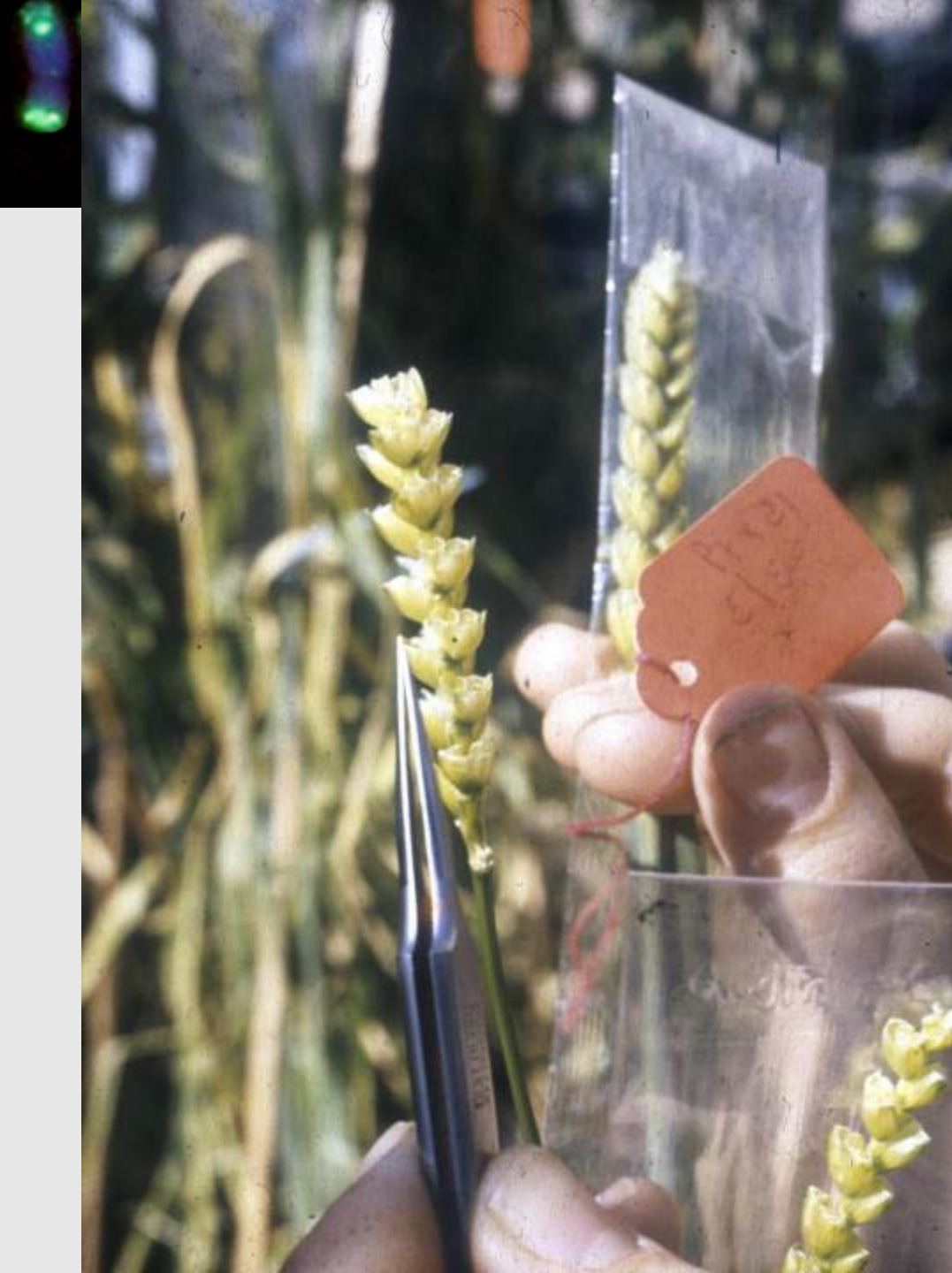


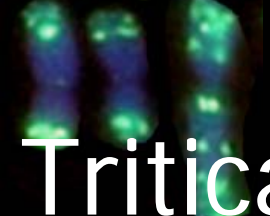
Allopolyploidy



Wheat evolution and hybrids







Triticale:
wheat x rye
hybrid



A decorative header image showing a series of chromosomes stained with green and blue fluorescent dyes against a black background. The chromosomes are arranged in a slightly curved line across the top of the slide.

Breeding strategies

AABBDD x RR

ABDR

Double chromosomes

AABBDDRR

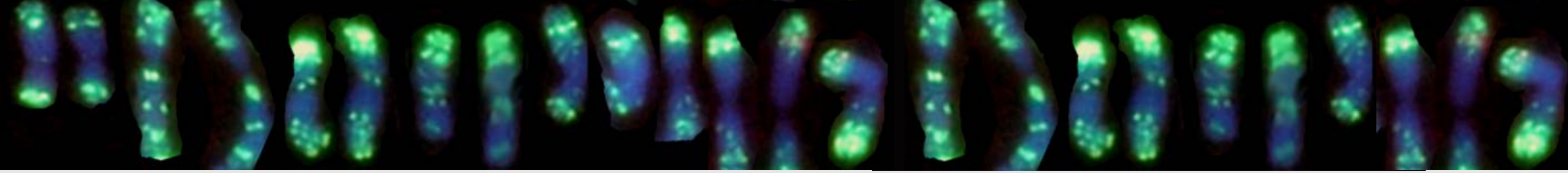
Backcross to wheat

Looses the R chromosomes eventually

AABBDD+1R

Induce recombination

AABBDD including 1BL/1RS



Total genomic DNA can be used as a probe to distinguish

- Genomes in sexual hybrids
- Alien chromosome introgression

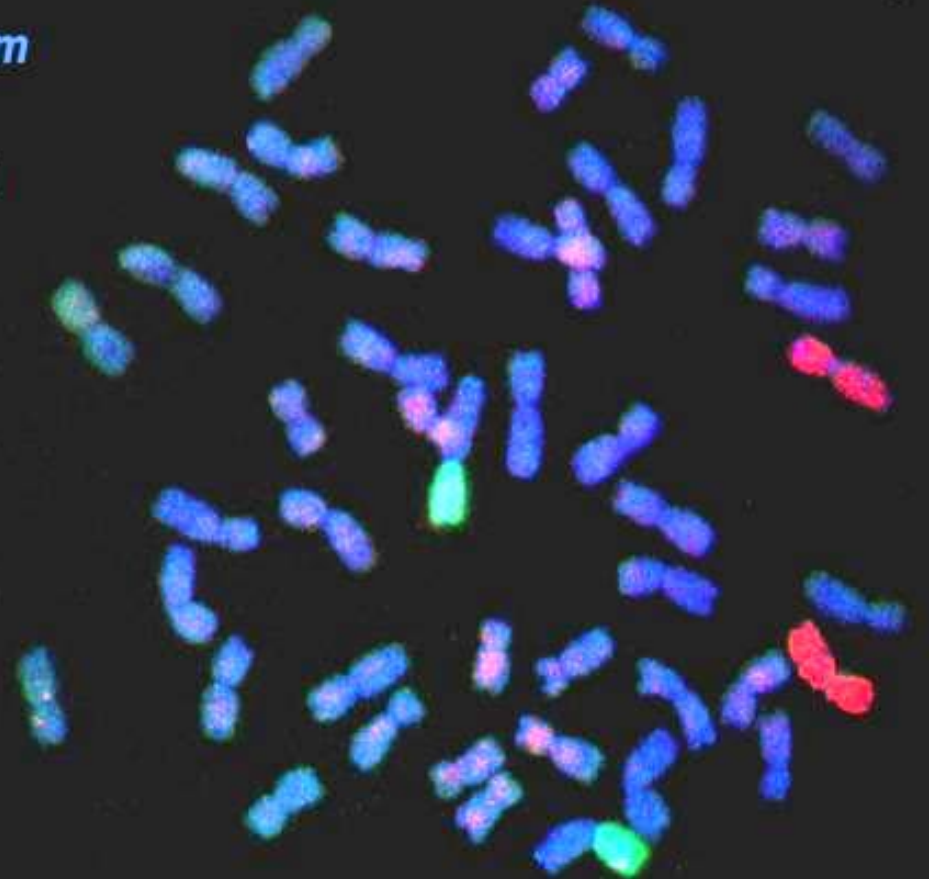
Triticum aestivum

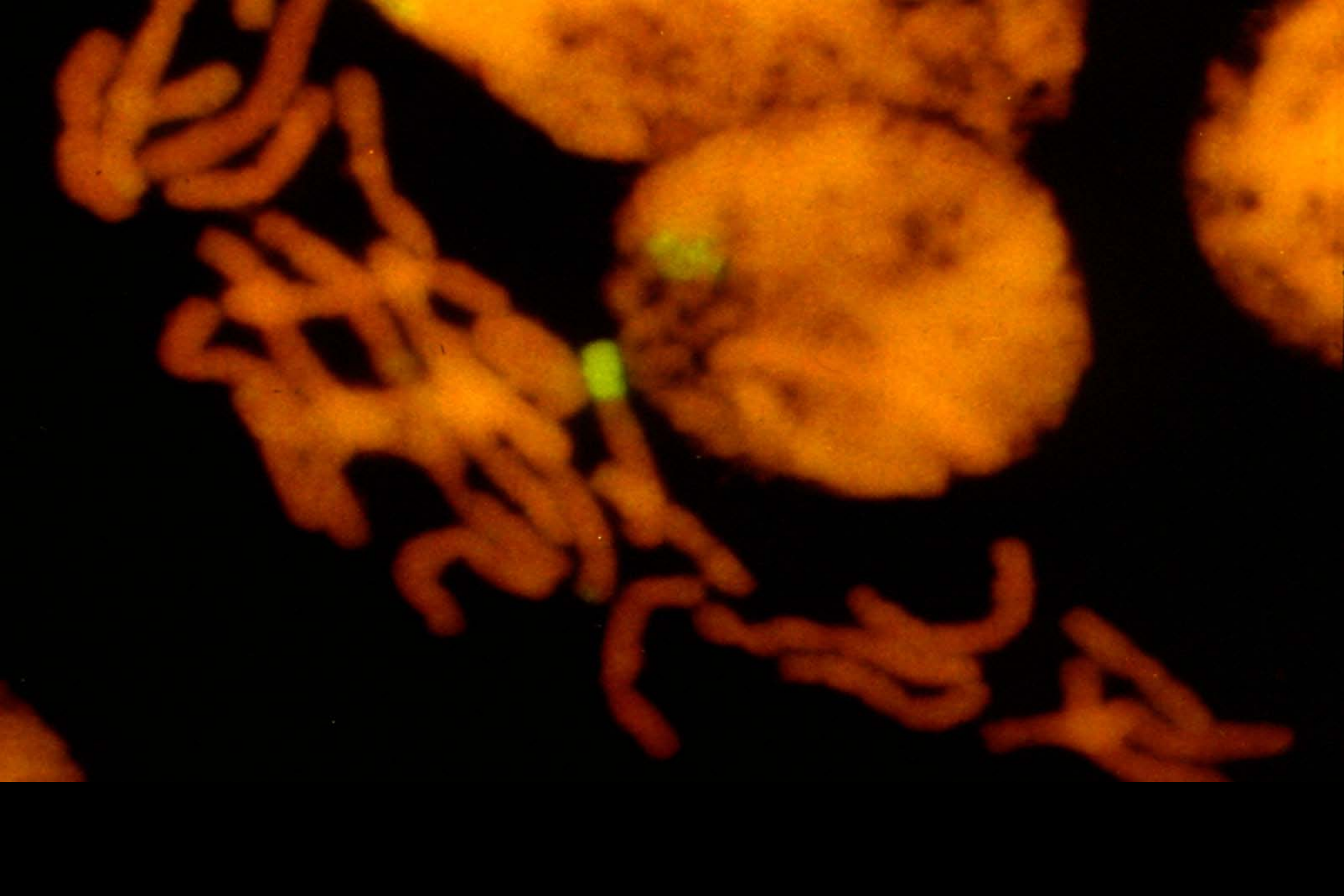
$2n=42+2$

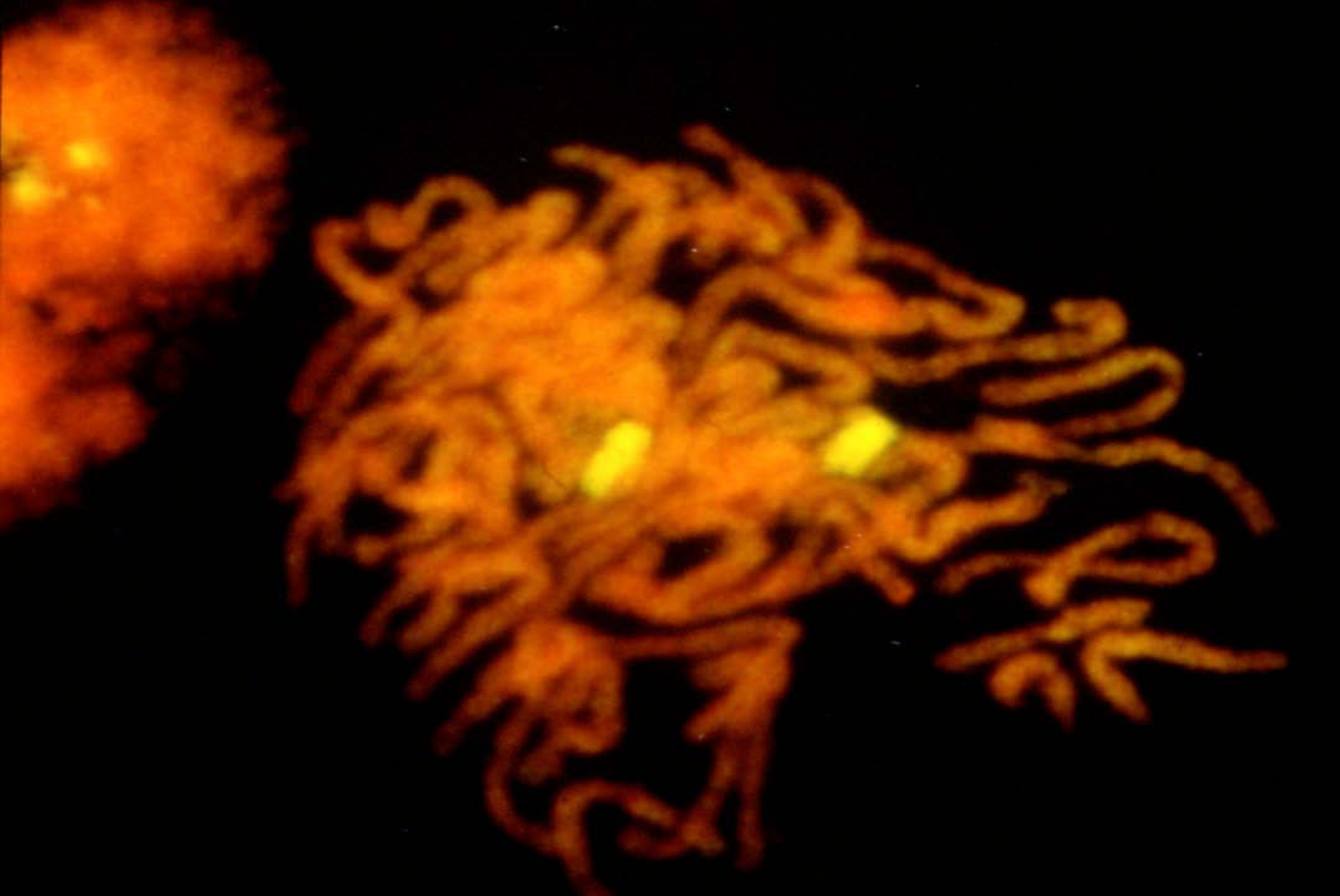
1B/1R translocation

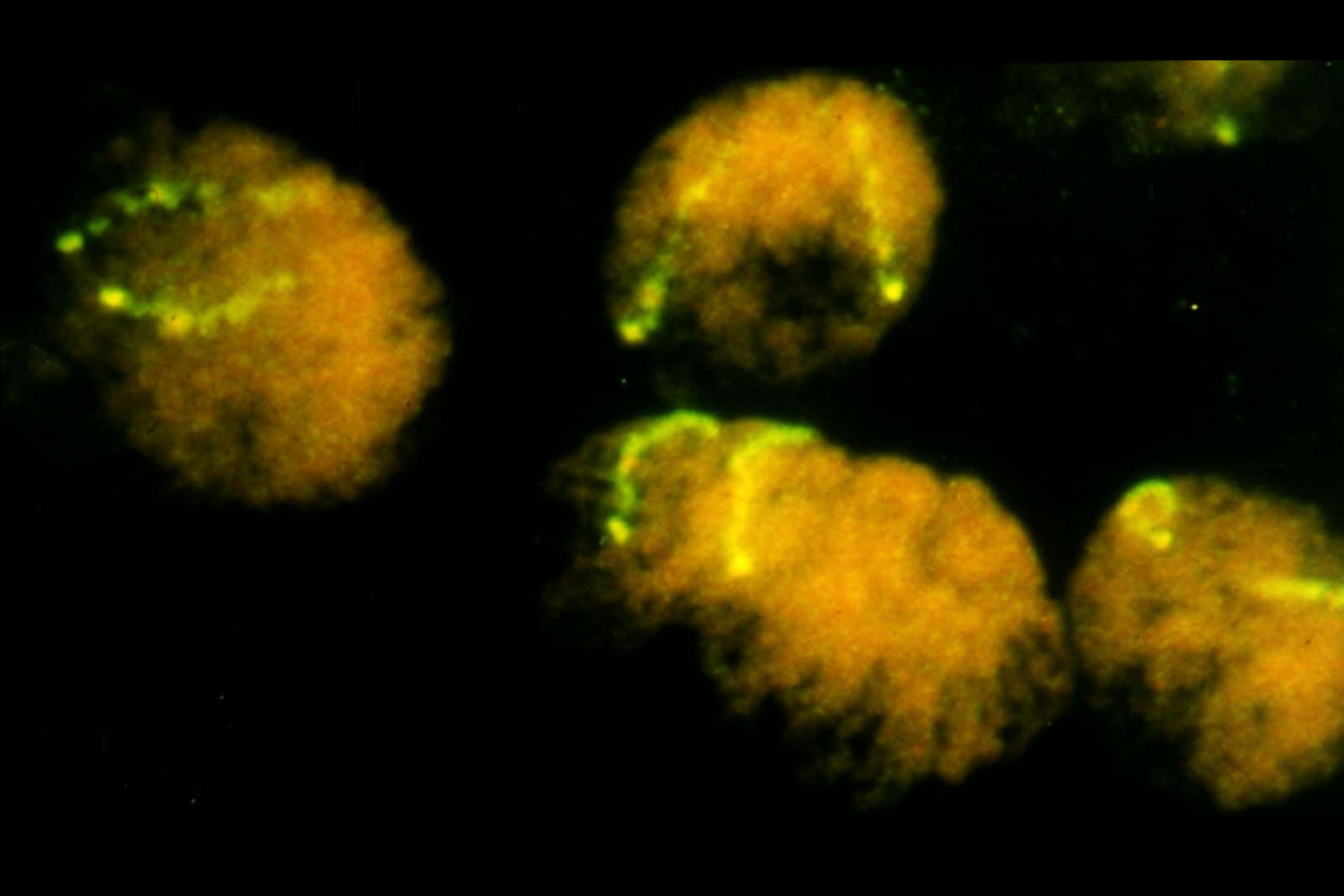
T. bessarabicum

addition





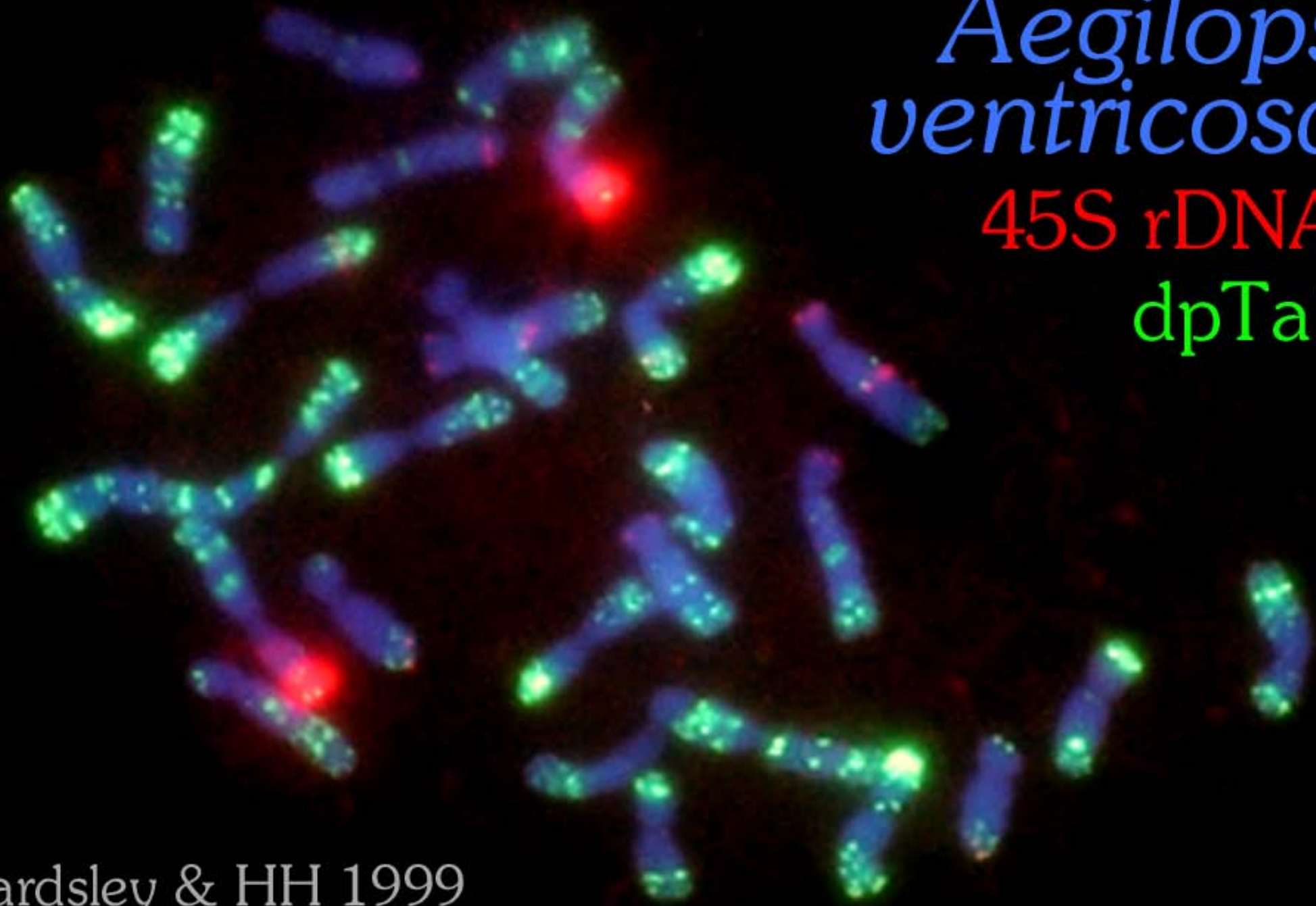


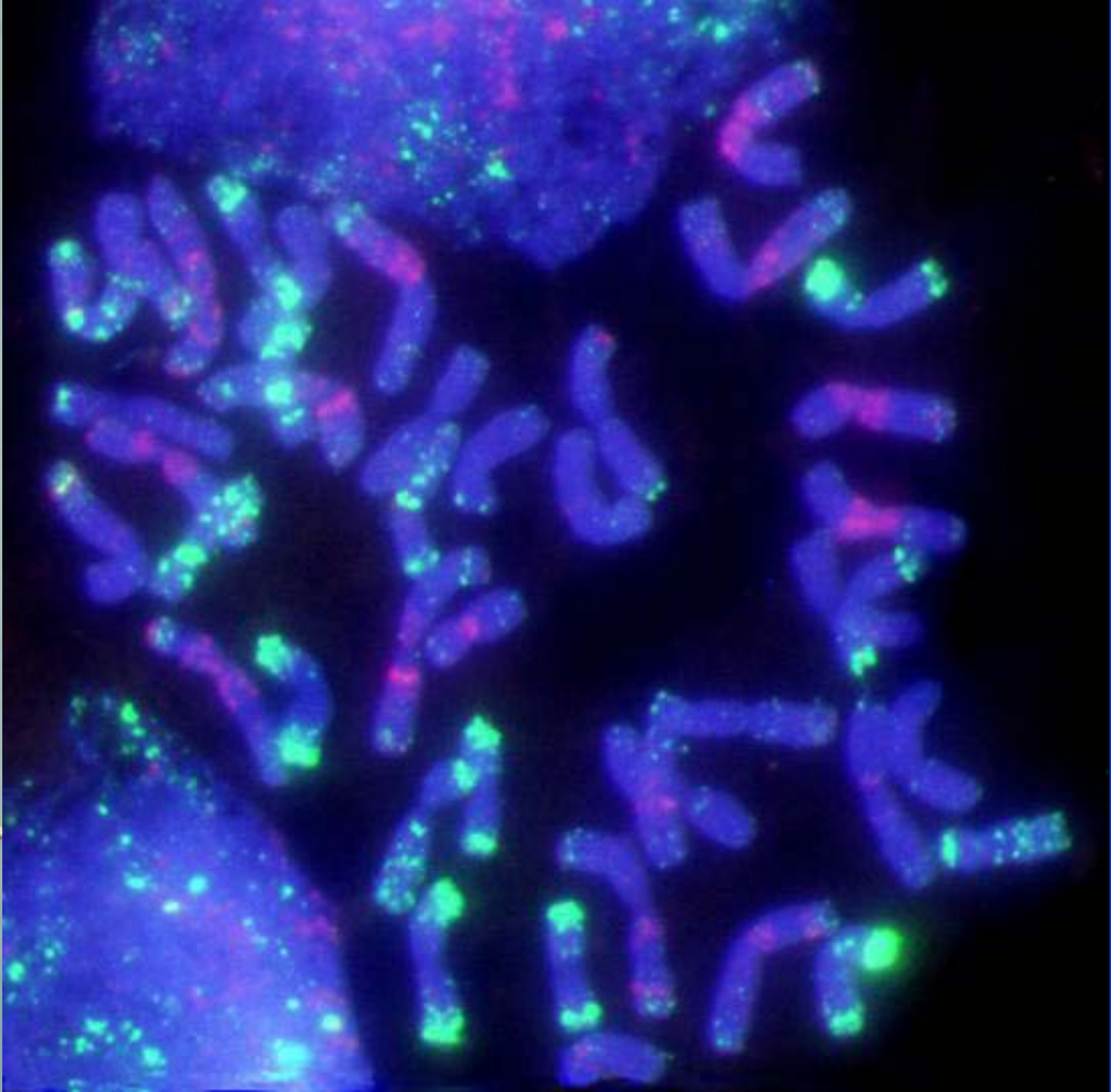


*Aegilops
ventricosa*

45S rDNA

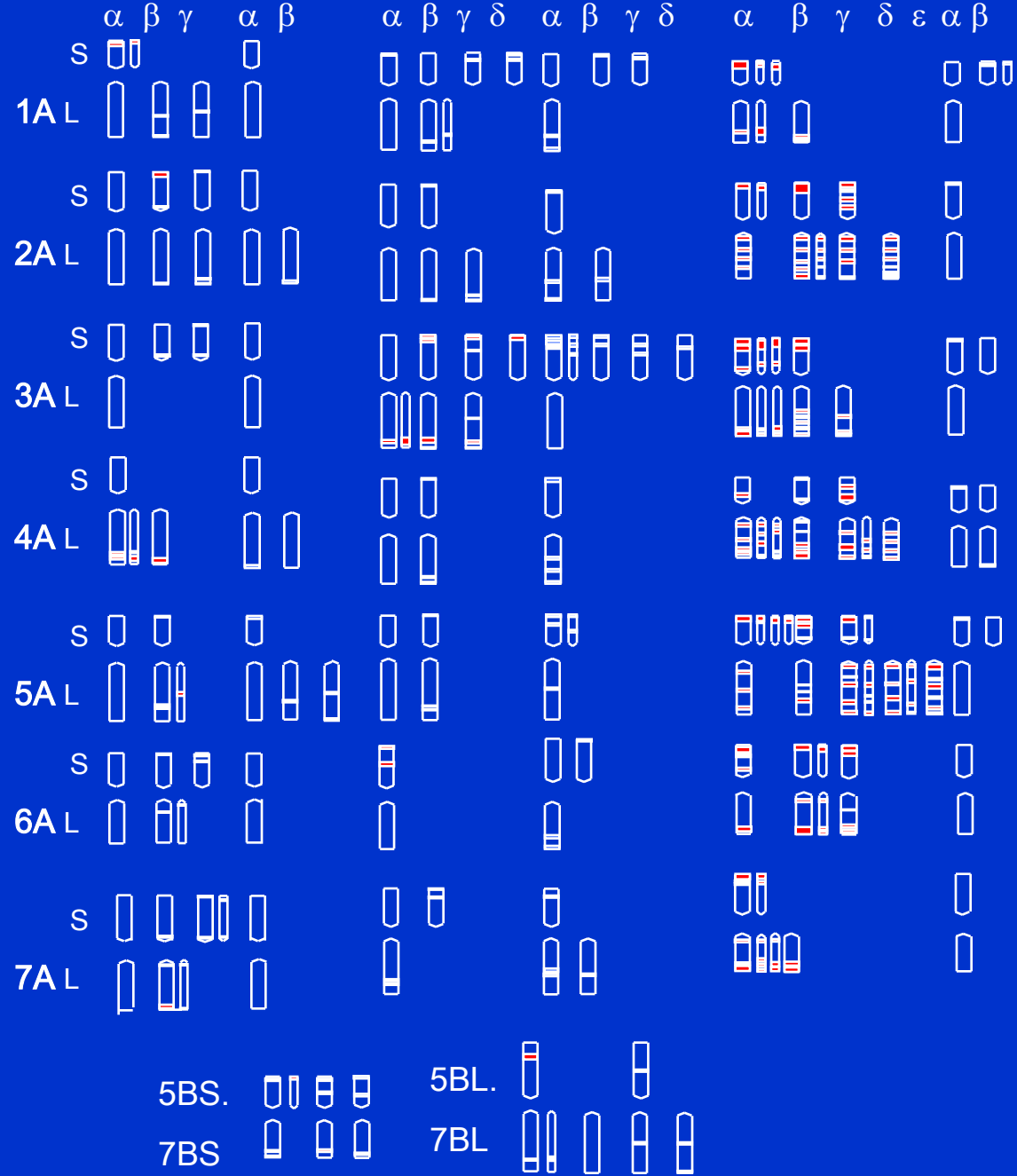
dpTa1





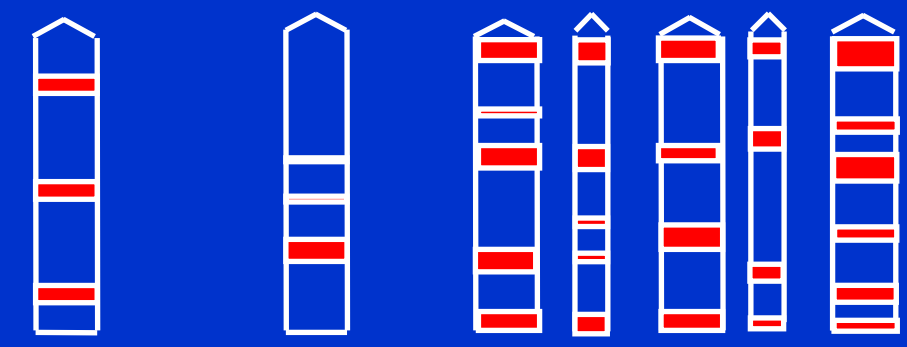


Lodging in cereals
UK July 2007



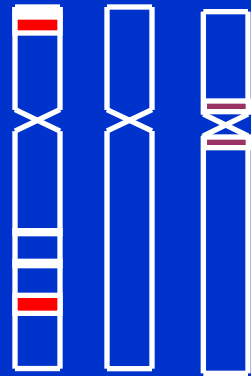
Multiple dpTa1 variants of each chromosome

e.g. 5DL



Inheritance of Chromosome 5D

Aegilops ventricosa
DDNN



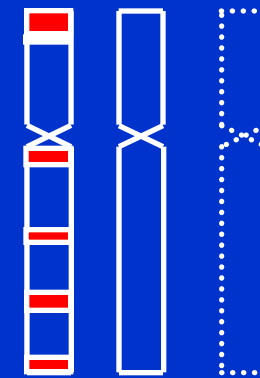
× *Triticum persicum* Ac.1510
AABB

ABDN



AABBDDNN

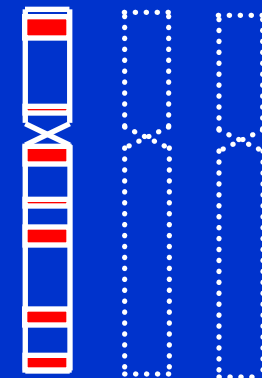
× Marne
AABBDD



VPM1



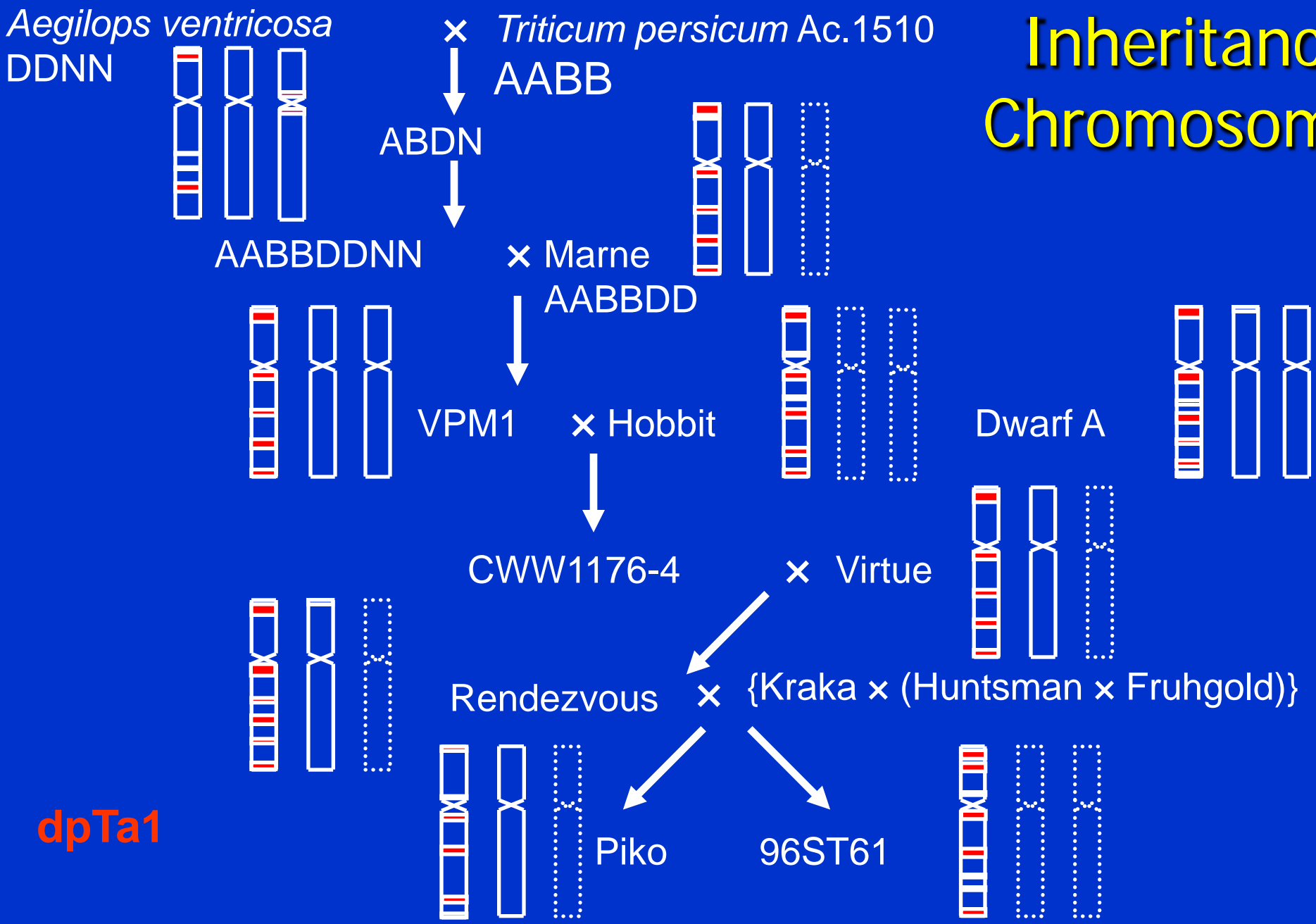
× Hobbit



CWW1176-4

dpTa1

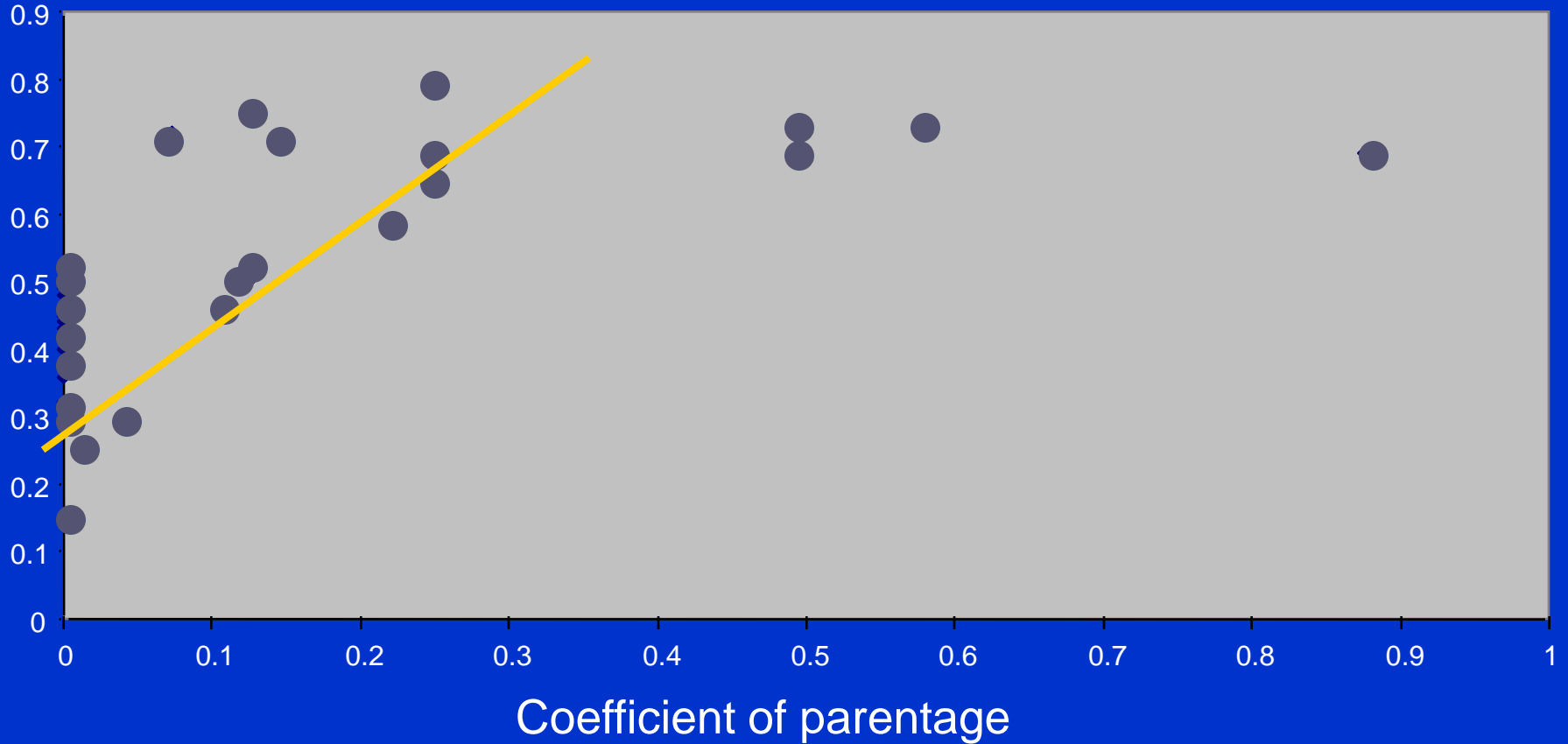
Inheritance of Chromosome 5D



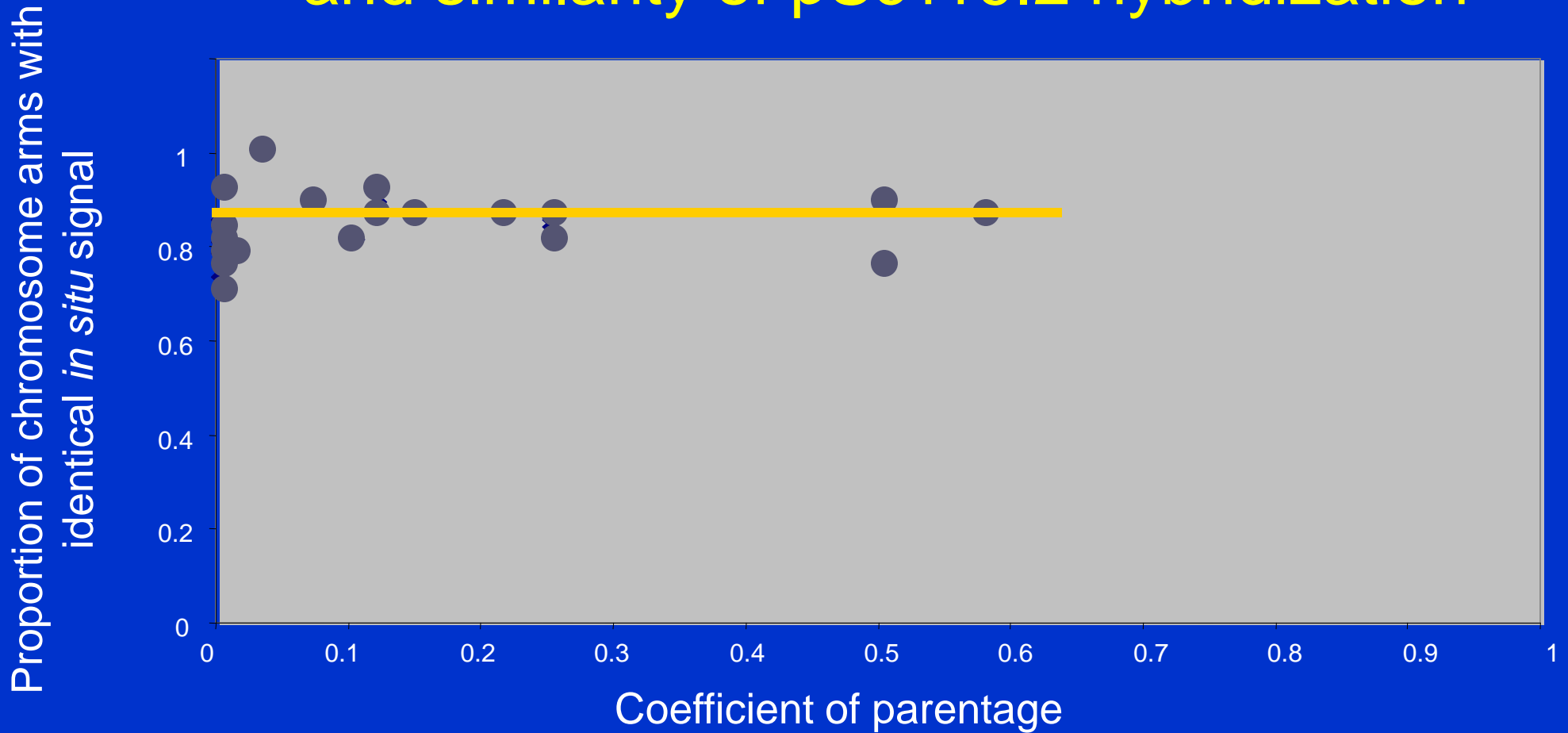
dpTa1

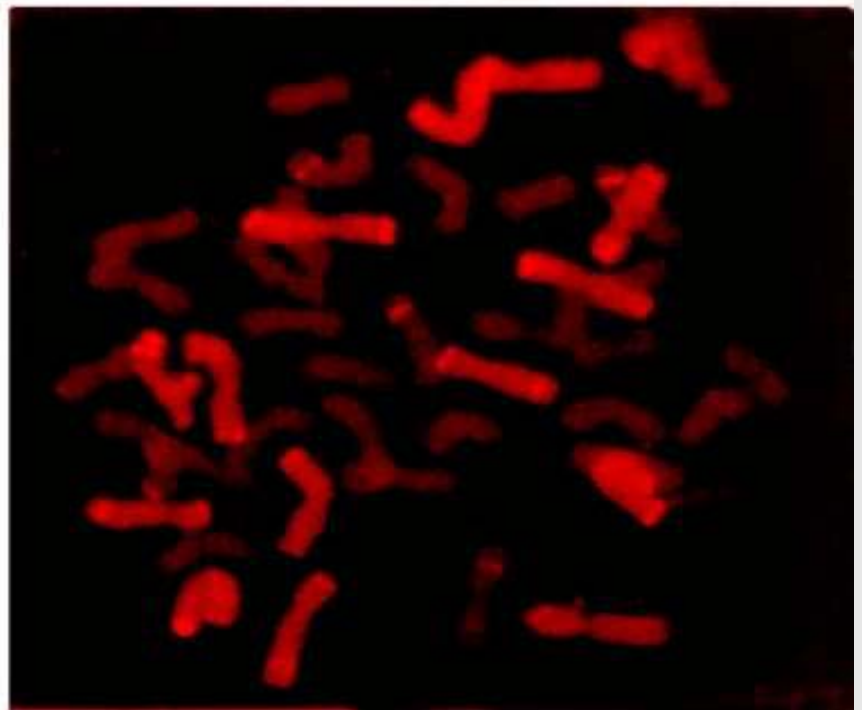
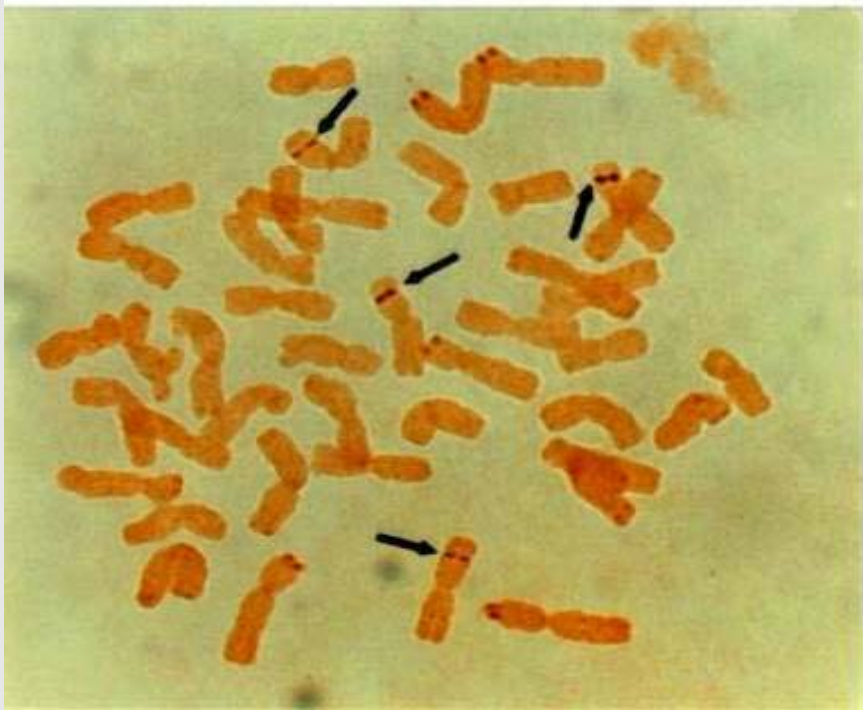
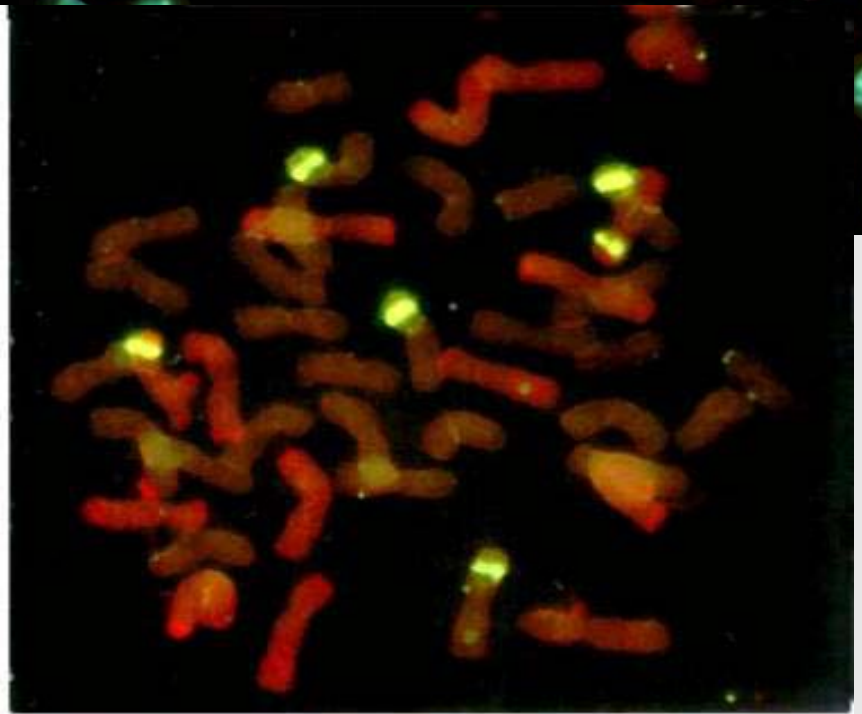
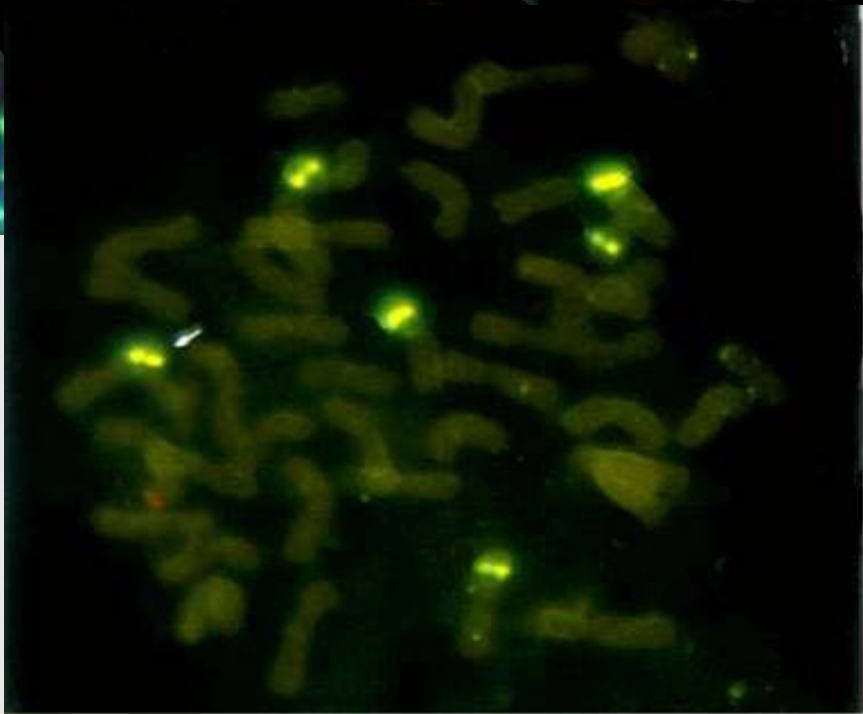
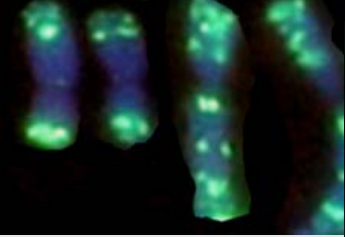
Correlation between genetic relationships and similarity of dpTa1 hybridization

Proportion of chromosome arms with identical *in situ* signal

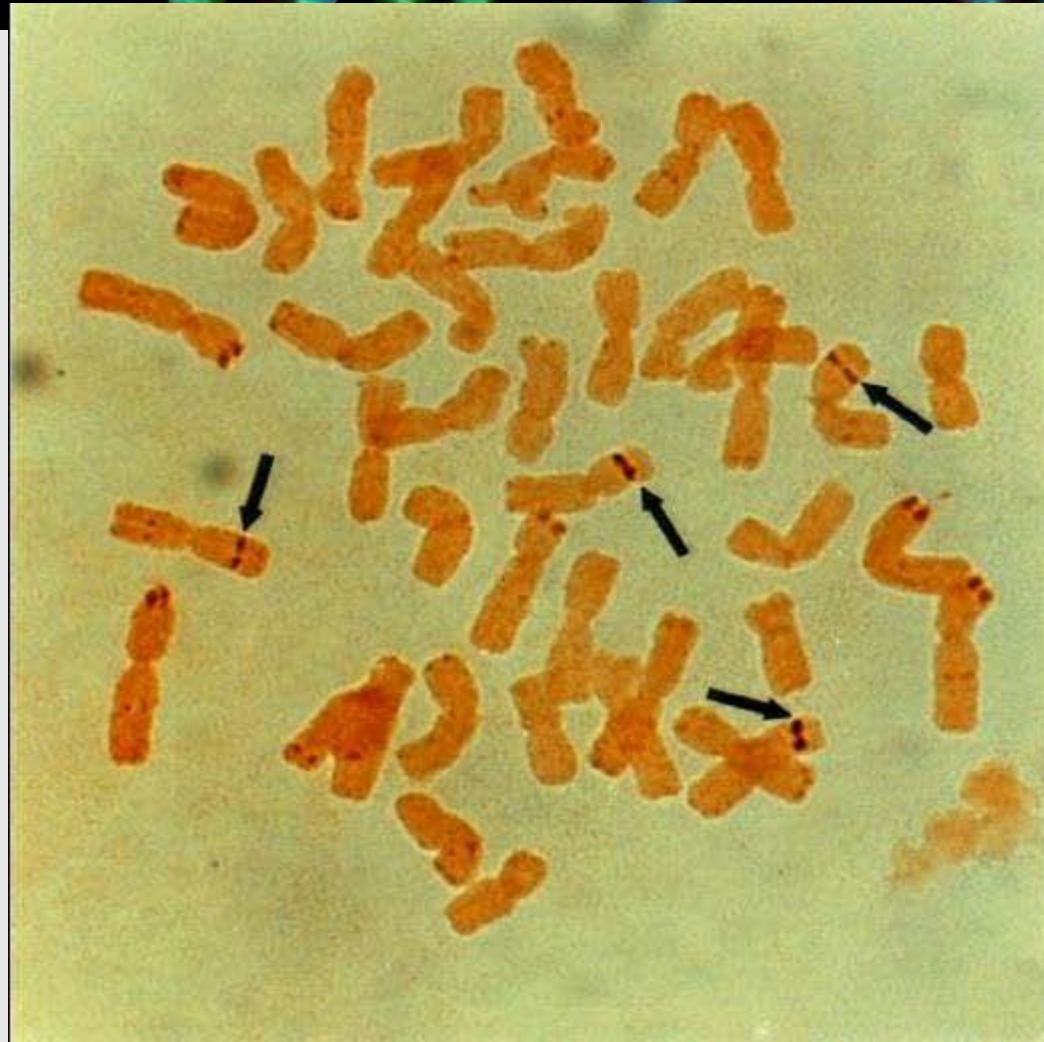


No correlation between genetic relationships and similarity of pSc119.2 hybridization

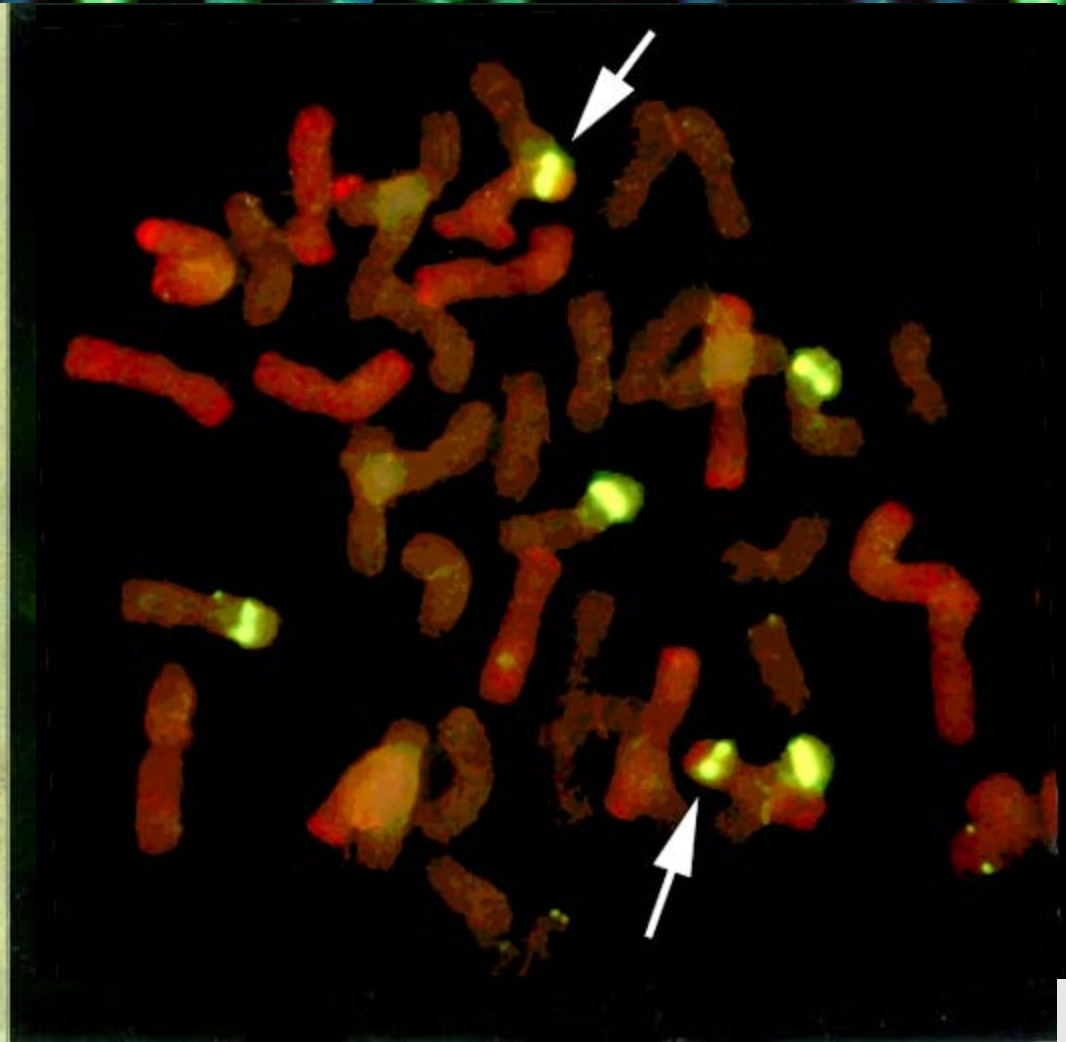




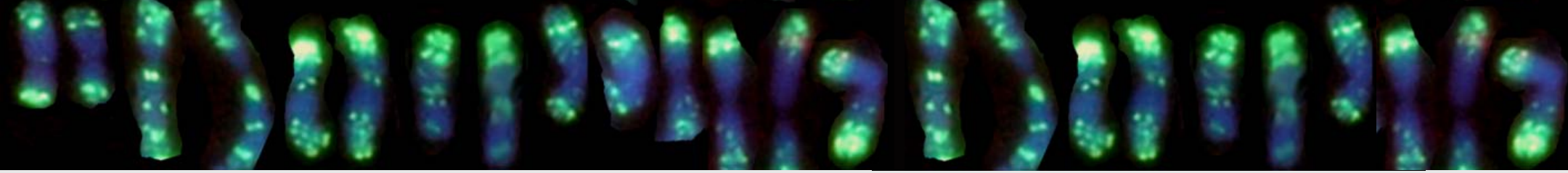
rRNA gene expression in Triticale



Four expression sites

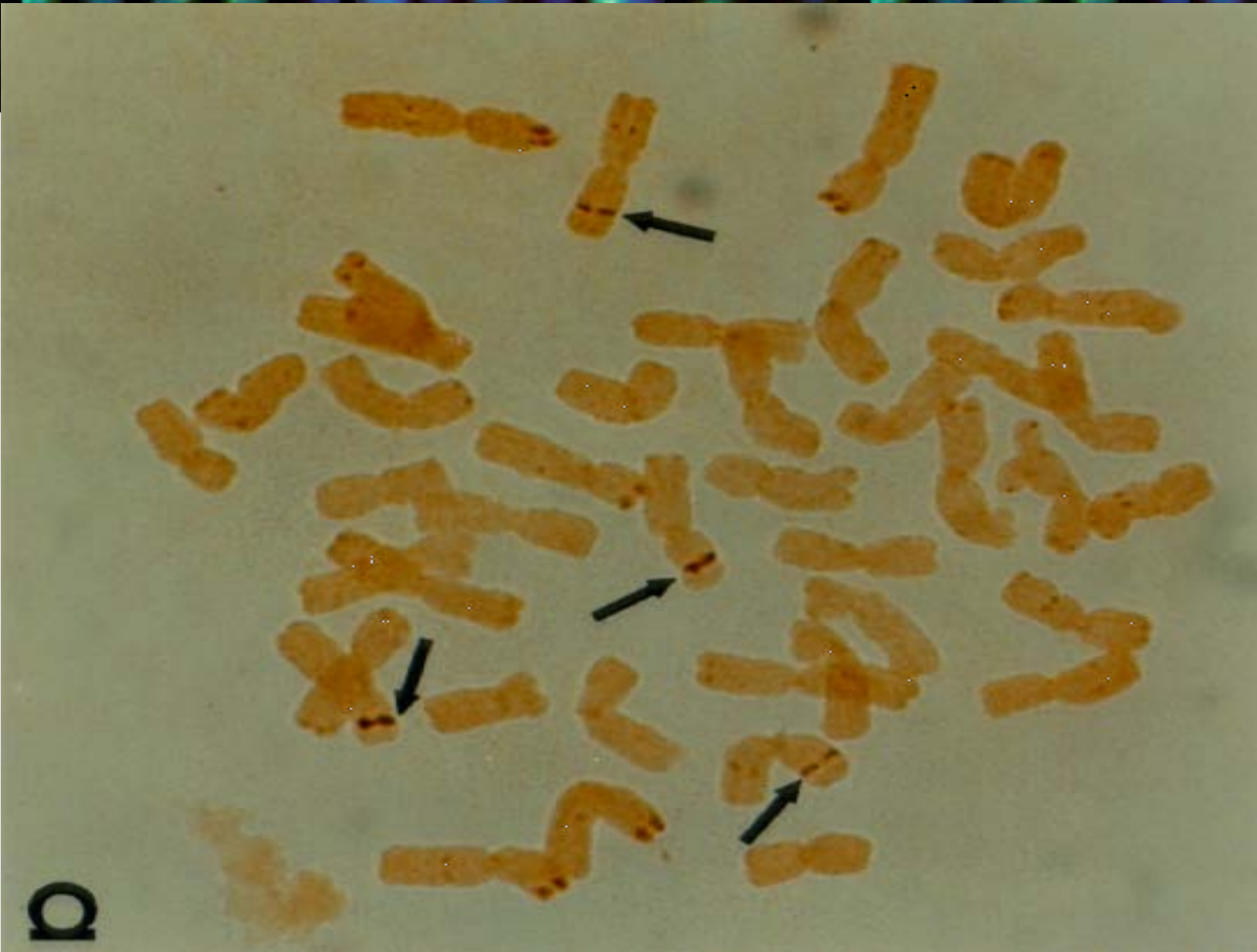
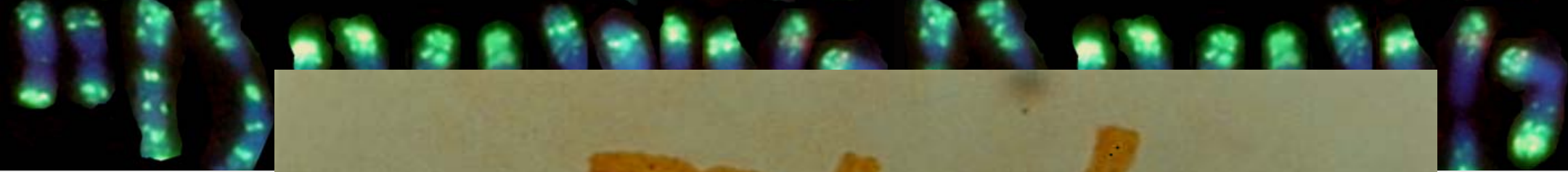


Six gene sites

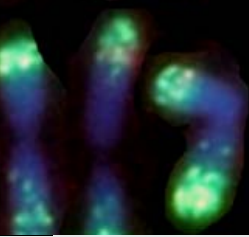
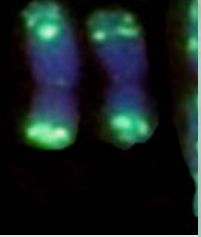


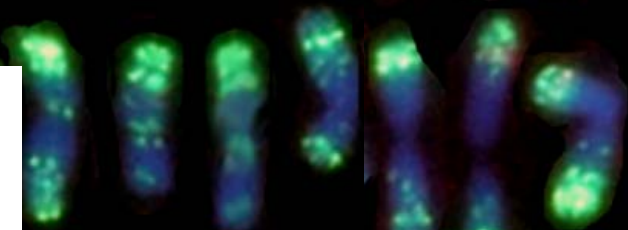
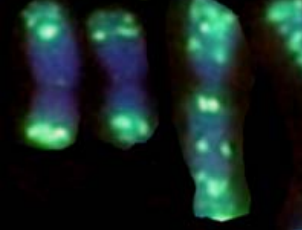
Modification of DNA Methylation

- ☞ Methylation widely implicated in gene expression control
- ☞ Treat with 5-azacytidine
 - N at carbon-5 position not C so $-\text{CH}_3$ cannot be added
- ☞ Effect of treatment on Triticale
- ☞ Ag-NOR method
 - see www.methods.molcyt.com methods page



a



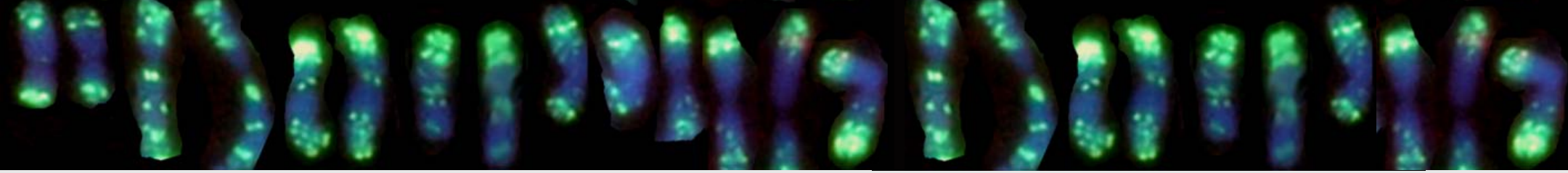


rDNA methylation
Probe
wheat rDNA rye rDNA spacer
Treatment
water AZC water AZC



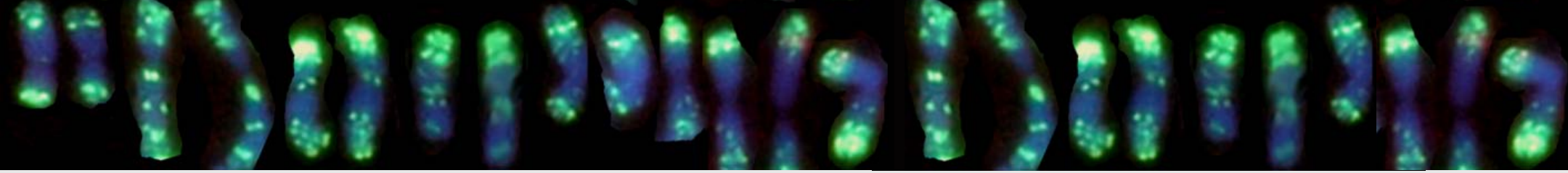
lambda
Apal
Drai
double
Apal
Drai
double

lambda
Apal
Drai
double
Apal
Drai
double



Modification of rDNA Methylation

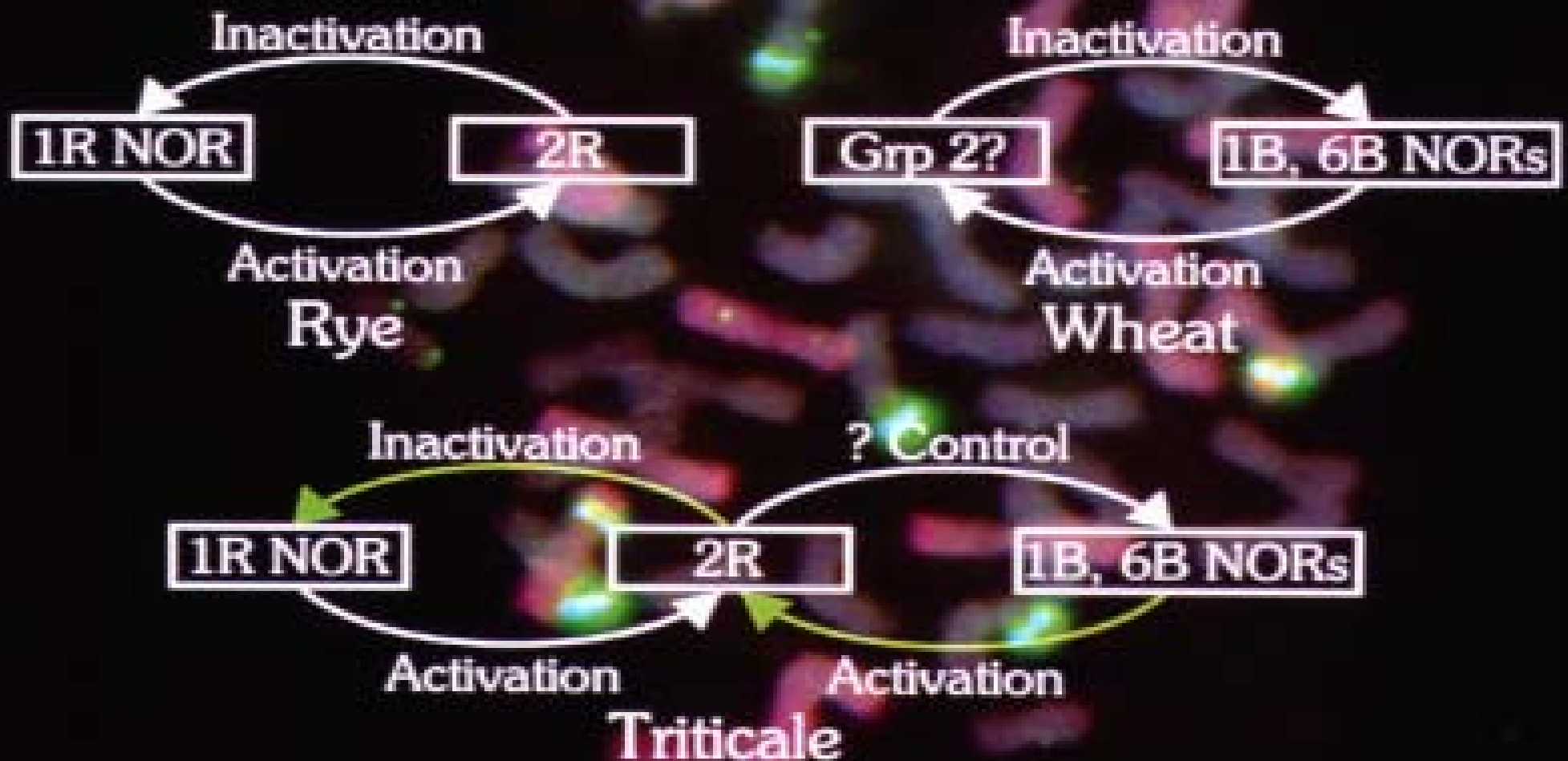
- ☛ Stability of methylation
- ☛ Seedling treatment – all 6 active for life
- ☛ Embryo treatment:
 - ☛ First 7 days – only wheat-origin active
 - ☛ After 7 days – rye and wheat-origin active



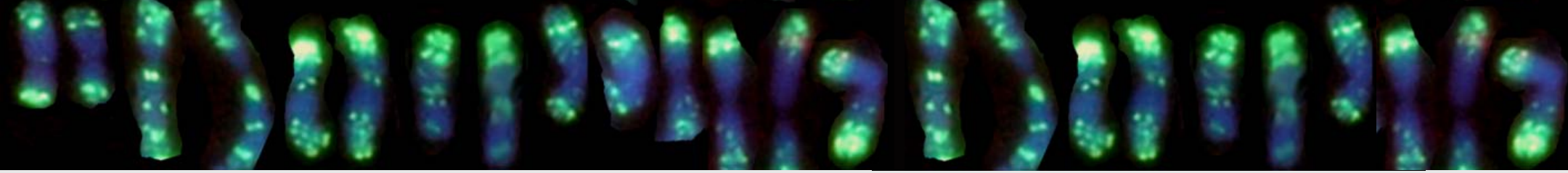
rDNA expression in Triticale

- ☞ Observation:
- ☞ Ag-staining showed 6 NORs sometimes
- ☞ These were in triticale with 12 rye chromosomes: 2D-2R substitution
- ☞ Found rather frequently so breeders must select it

Interactions between rDNA and control loci







Aegilops tauschii (D genome donor) in Iran

57 accessions collected

● *ssp. tauschii*

- *var. meyeri* (18)
- *var. tauschii* (22)
- *var. anathera* (4)
- *var. meyeri* (12)



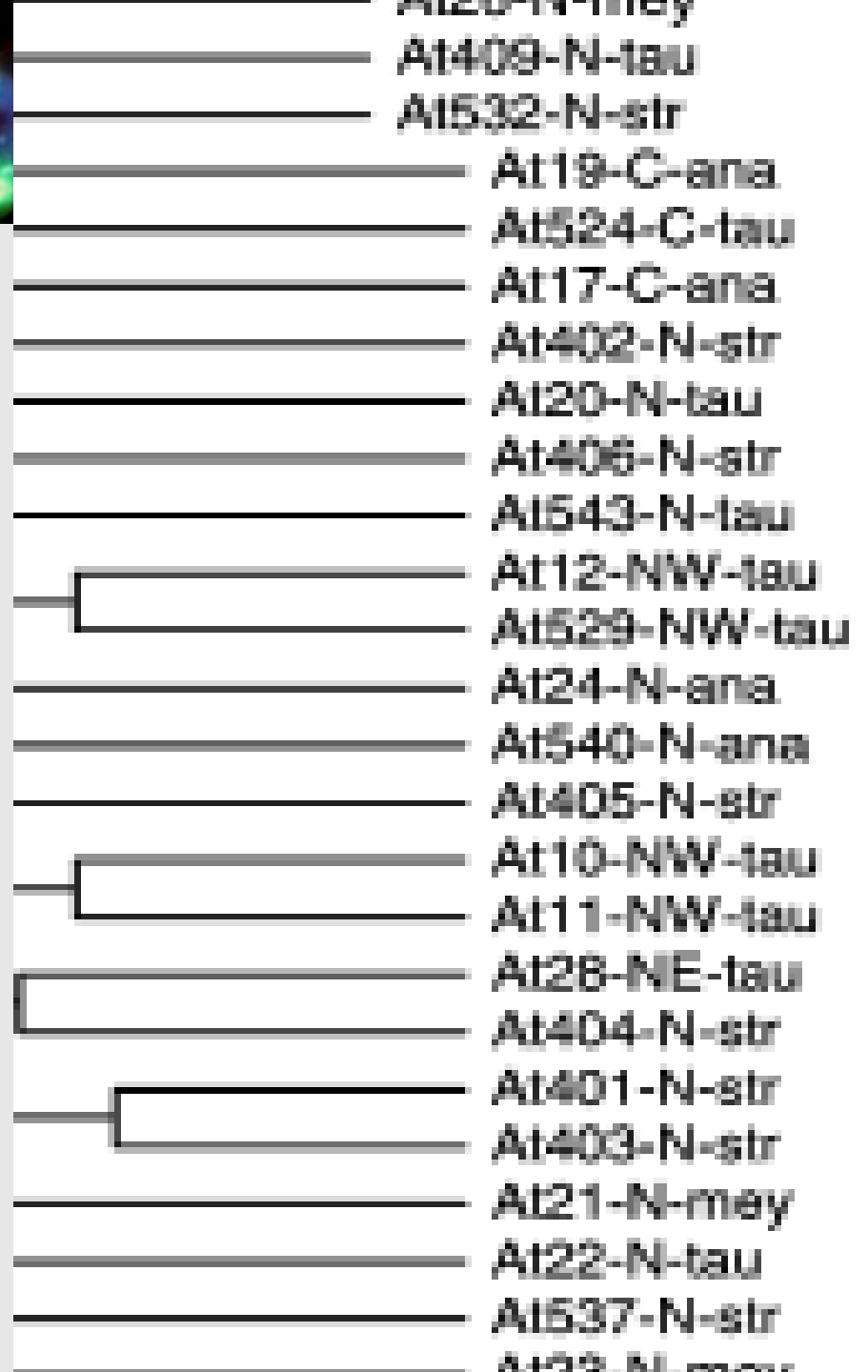
Hojjatollah Saeidi, Mohammad Reza Rahiminejad, Sadeq Vallian, HH
Genetic Resources & Crop Plant Evolution 2006



Diversity in D genome

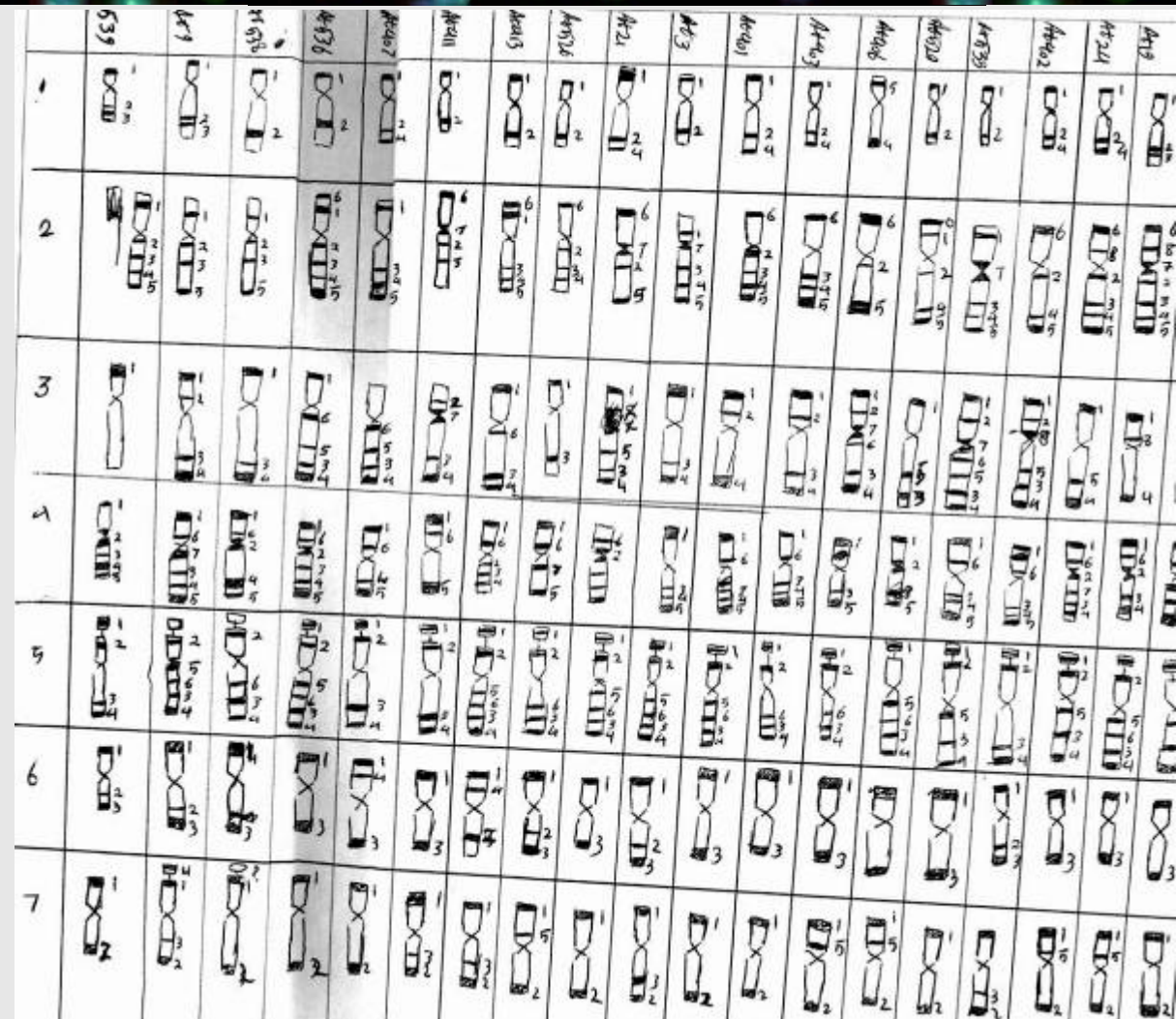
- Microsatellite markers
- 57 accessions of wild *Aegilops tauschii* ($2n = 2x = 14$; D genome)
- No SSR markers were characteristic for taxa or geographical origin
- High diversity present

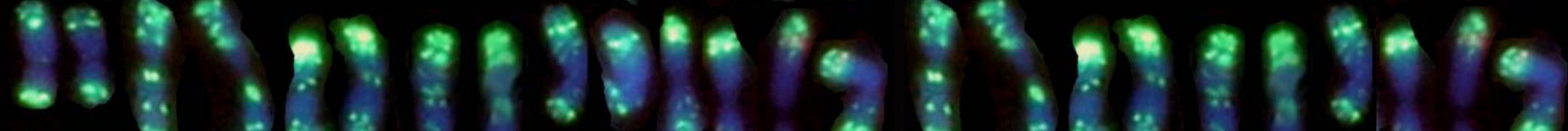
Saeidi, HH et al. Genet Resources & Crop Evolution 2005



Aegilops tauschii in Iran

dpTa1-Repetitive
banding pattern
does correlate
with taxonomic
grouping



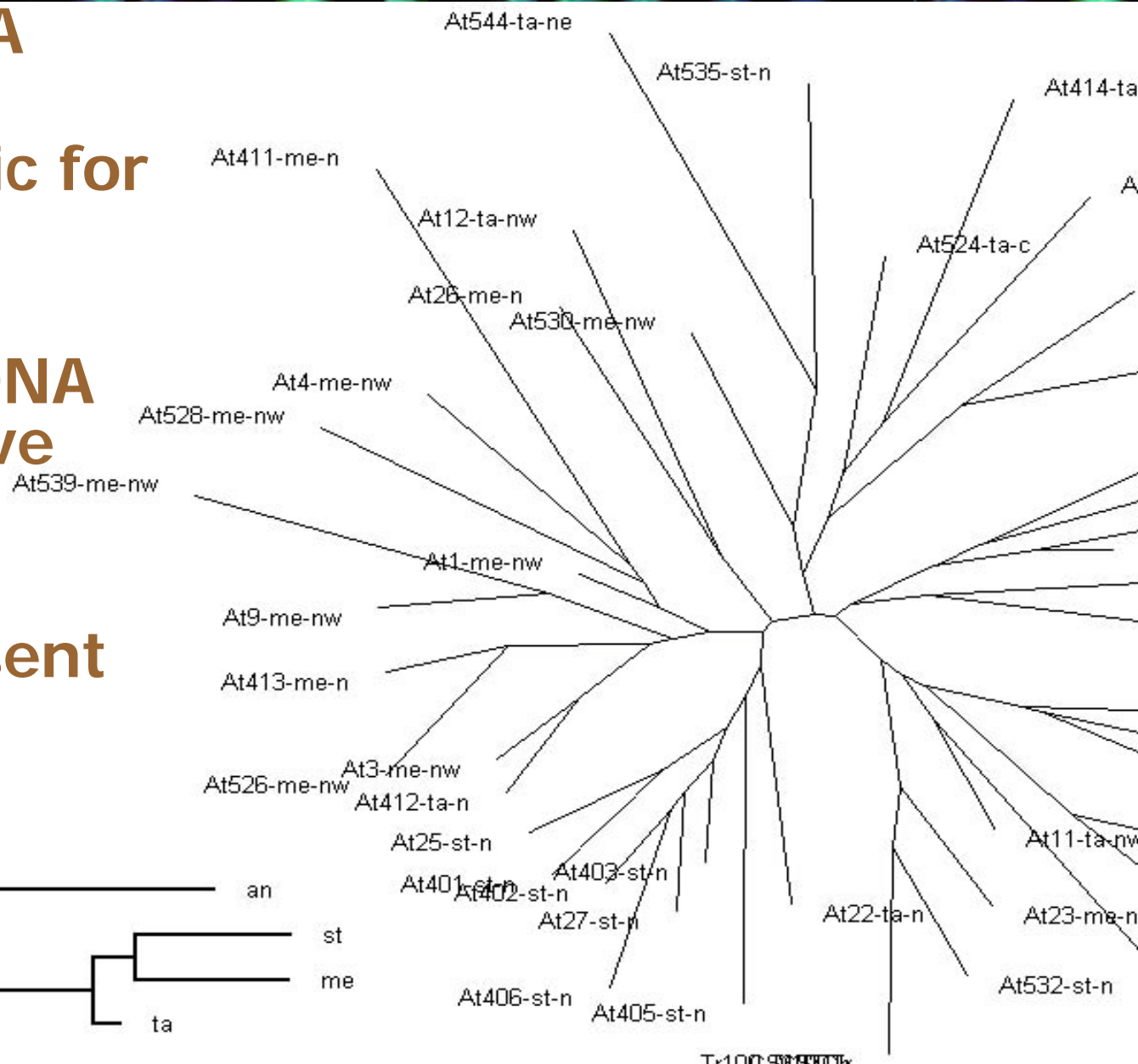


In situ repetitive DNA markers

Markers characteristic for taxa

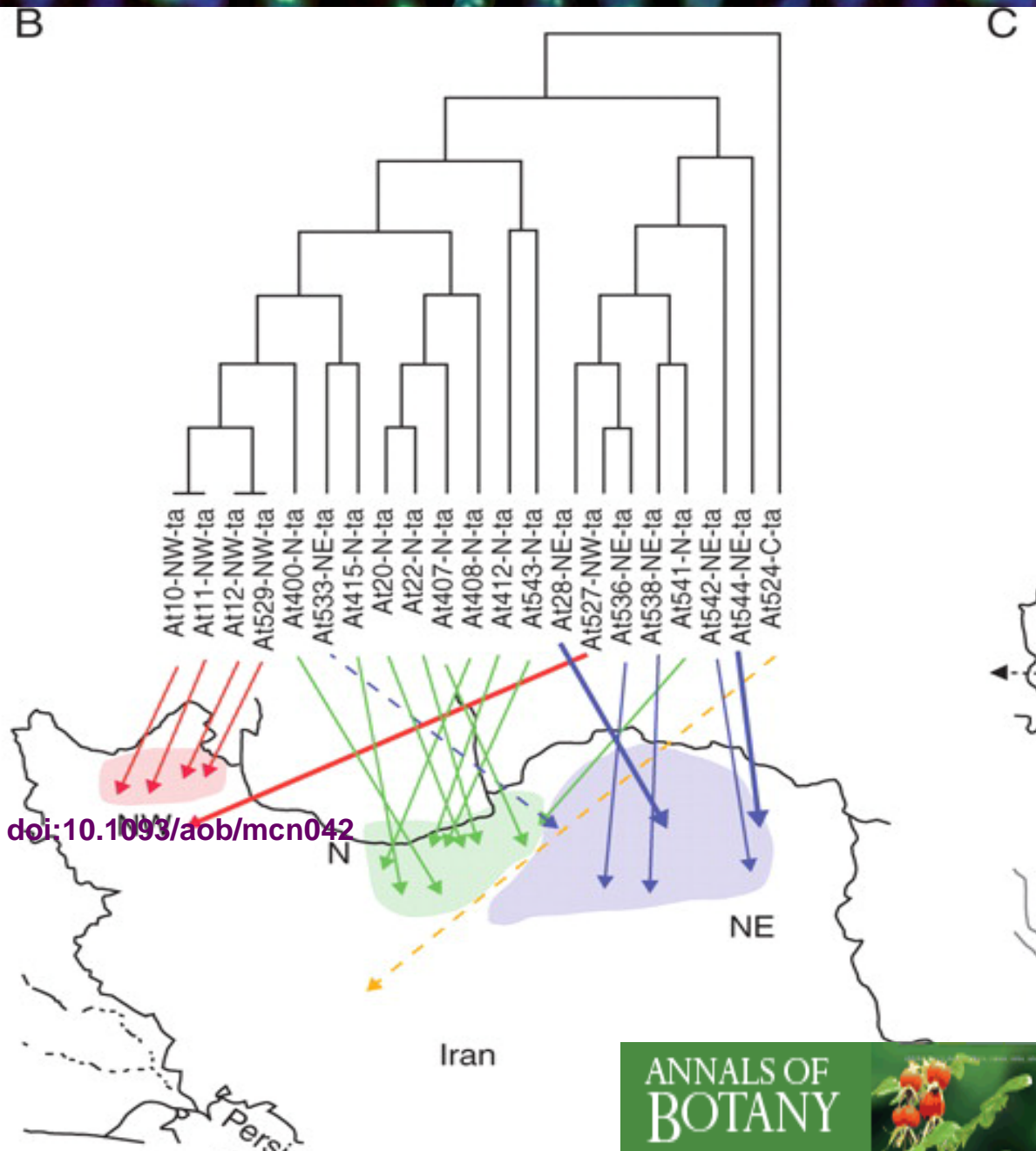
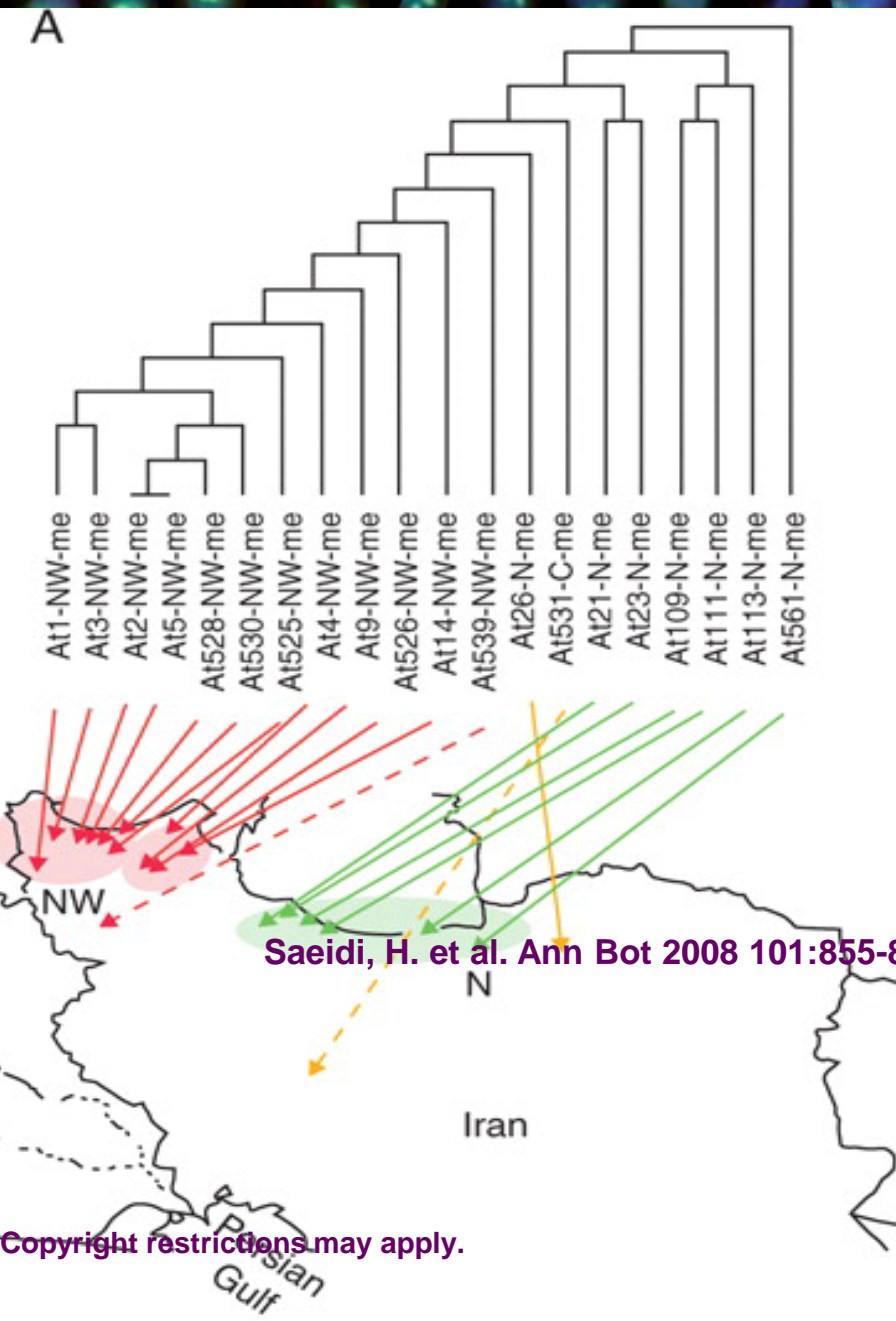
Evolution of genes/DNA markers and repetitive (SSR are different)

High diversity present
Useful genes for wheat breeding



Tr100-SSR

UPGMA dendrograms of the relationships based on IRAP analysis of (A) accessions of *Ae. tauschii* subsp



Saeidi, H. et al. *Ann Bot* 2008 101:855-861; doi:10.1093/aob/mcn042

Copyright restrictions may apply.



Demonstration of the direction of distribution (phylogeography) even over short geographic distances

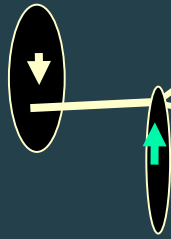
Phylogeography of *Ae. tauschii*
Species originated from North of Iran and distributed in two directions.

tauschii genotype passes from middle parts of Alborz Mountains and the distributed eastward and westward (direction 1)

strangulata genotype are distributed along the Caspian Sea shore (direction 2)



Cross-pollinating
ancestor



FISH

IRAP

susp.
strangulata

SSR

var. *meyeri*

var. *anathera* subsp.
tauschii

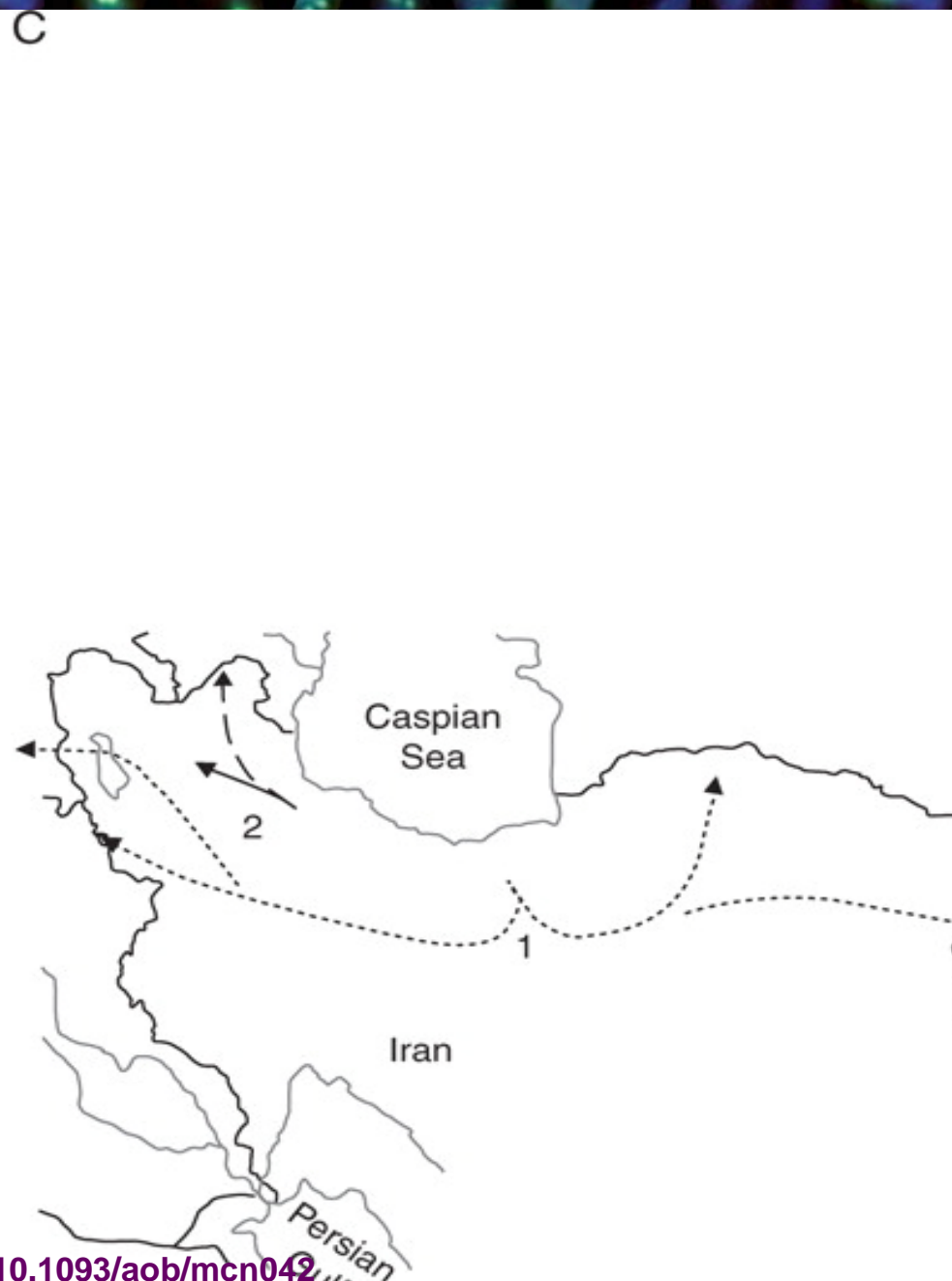
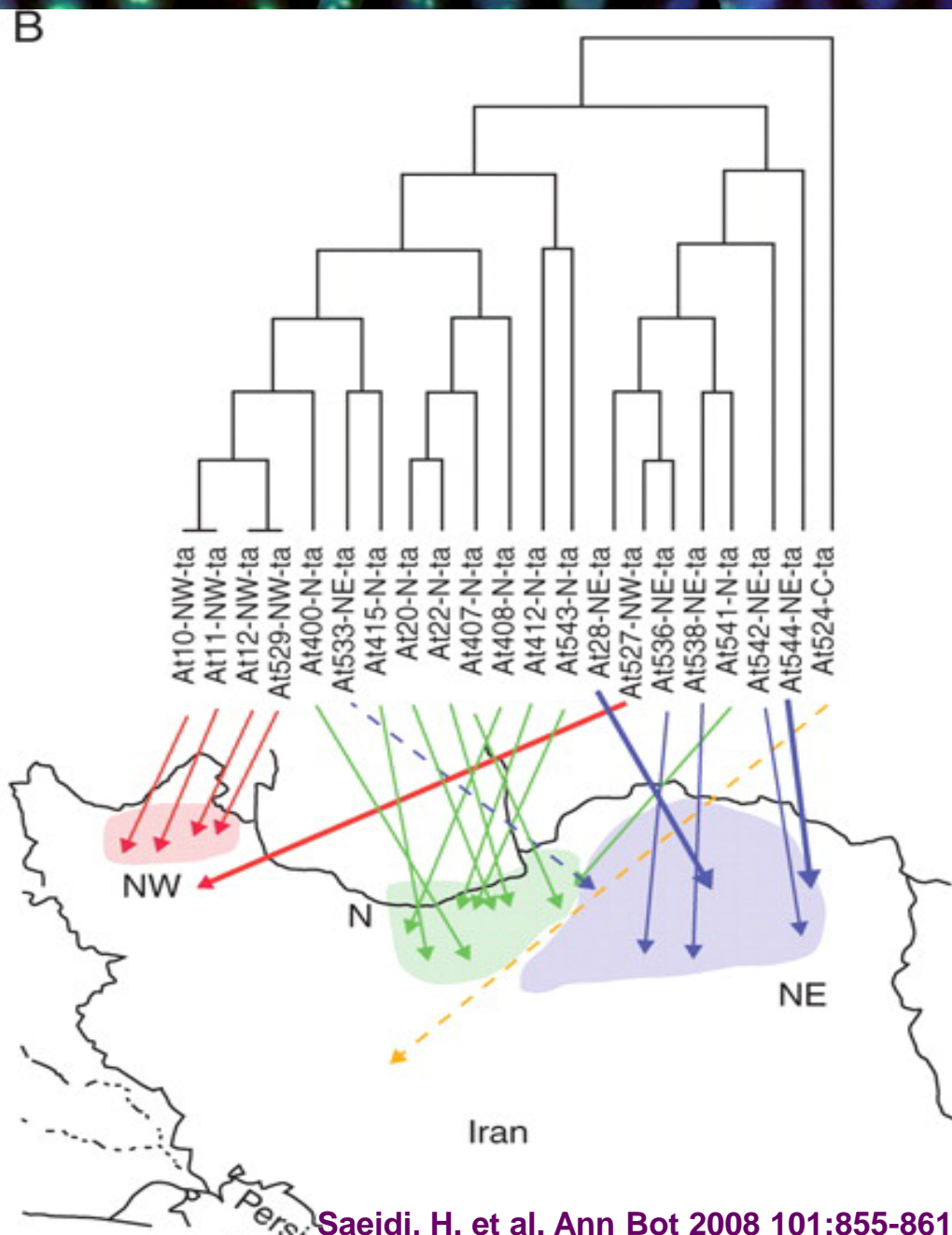
var. *tauschii*

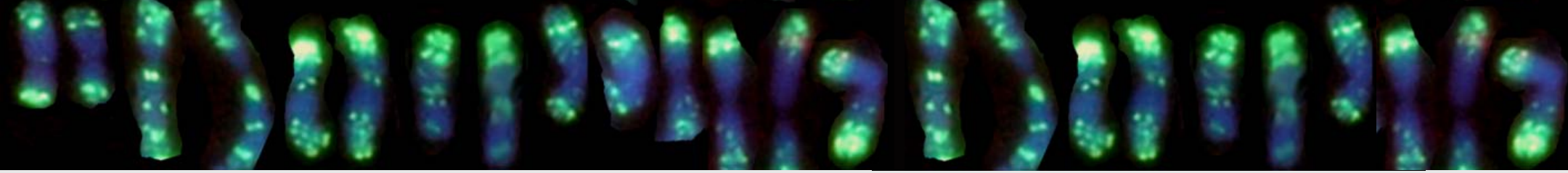
Self-pollinating
ancestor

(Aegilops tauschii)

*An evolutionary model
supported by molecular
analyses*

UPGMA dendrograms of the relationships based on IRAP analysis of (A) accessions of *Ae. tauschii* subsp



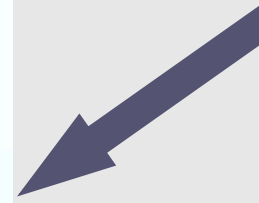
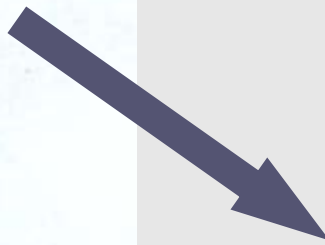


- Different DNA sequences evolve more-or-less independently
- There is no 'molecular clock' for many sequence types
- Multiple sequence types should be used to gain a full understanding of genome evolution

- Wild Wheat – Using Genes -

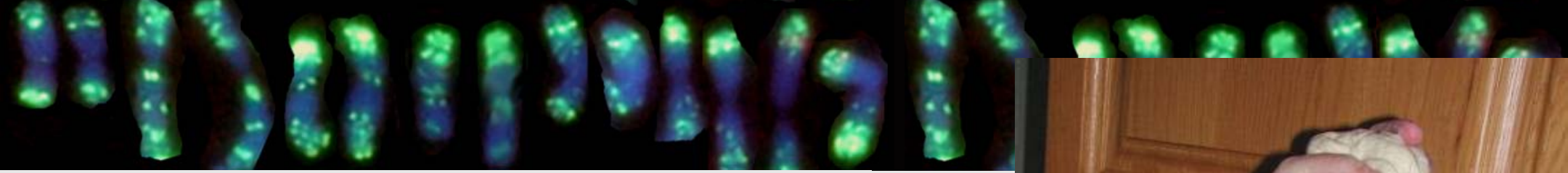
Aegilops

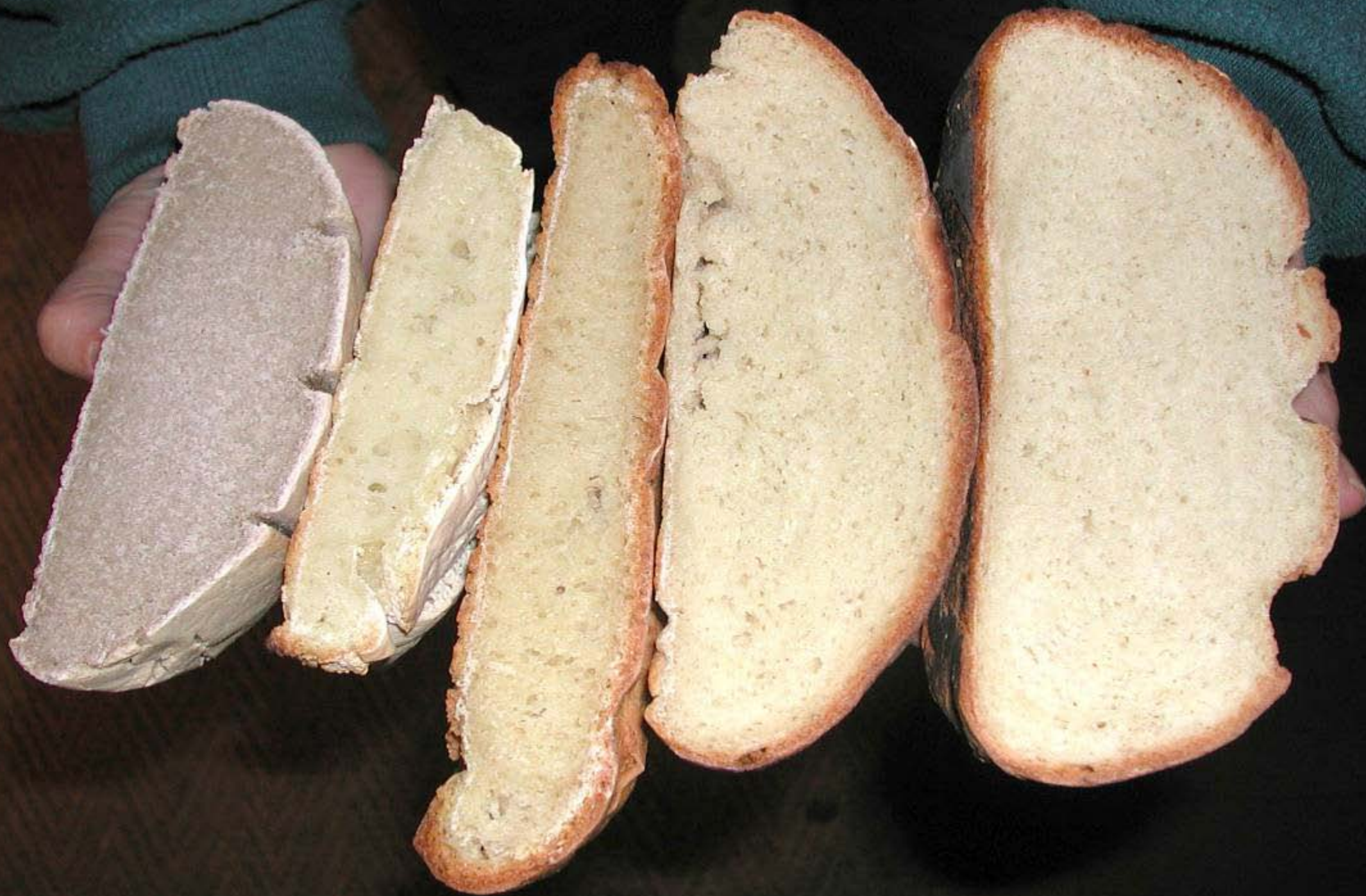
Triticum durum



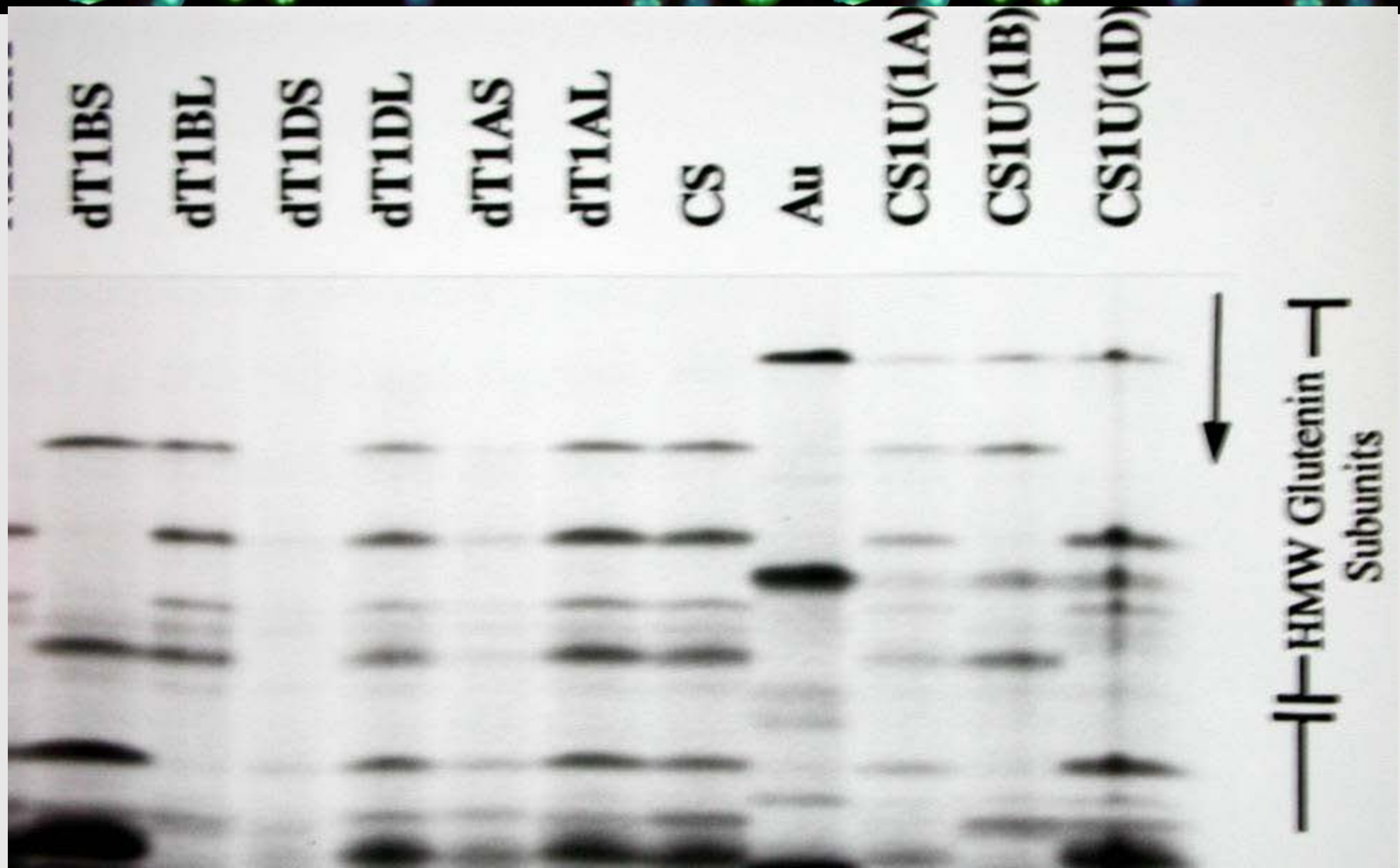
C0175 Espanhol 8914

Ae. umbellulata type × *T. durum*





Wheat proteins: High bands = good bread

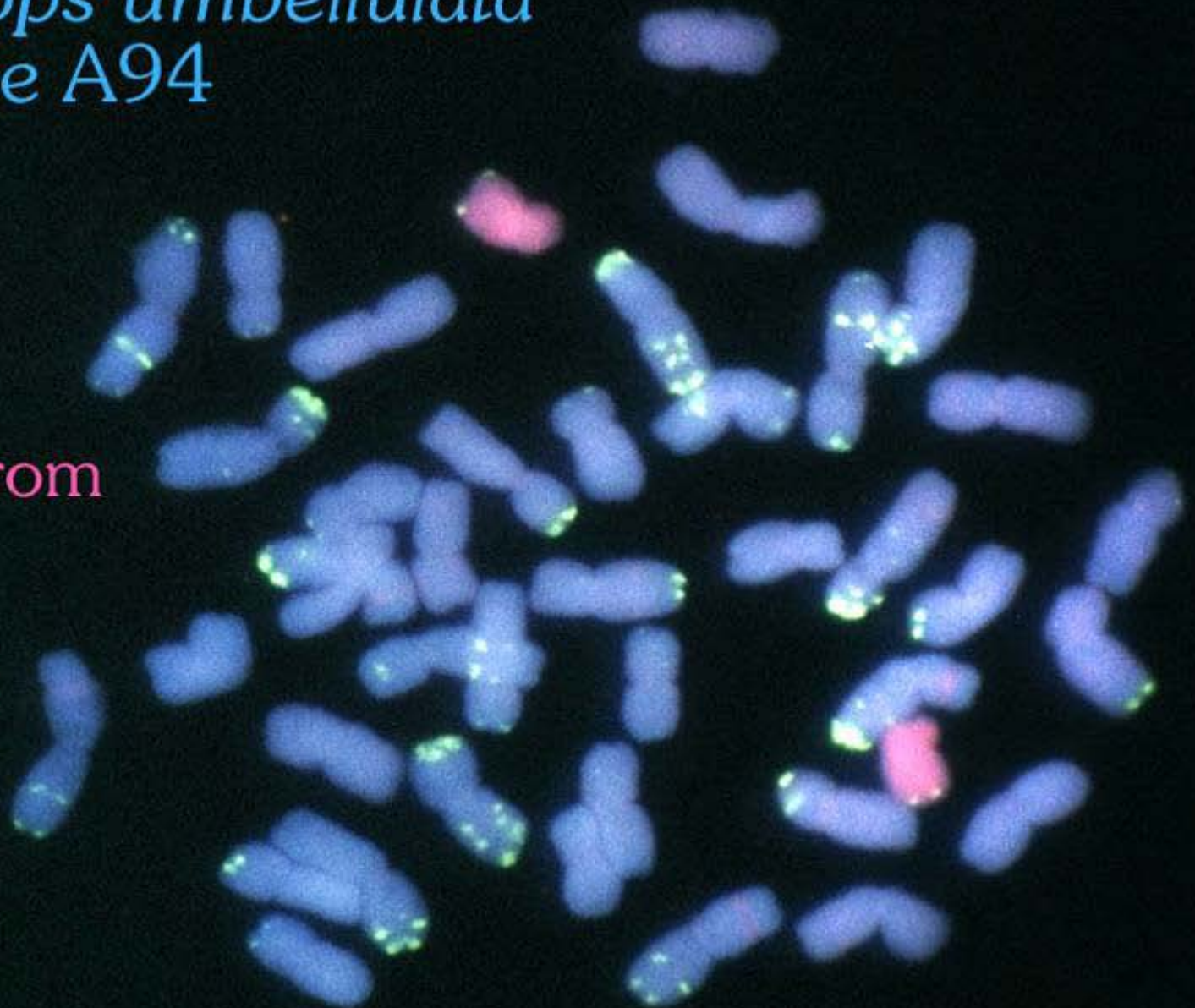


Wheat - *Aegilops umbellulata*
substitution line A94

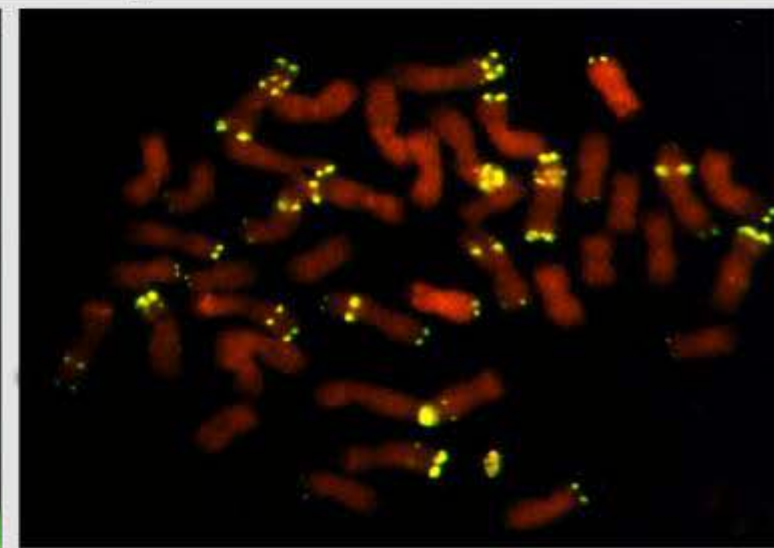
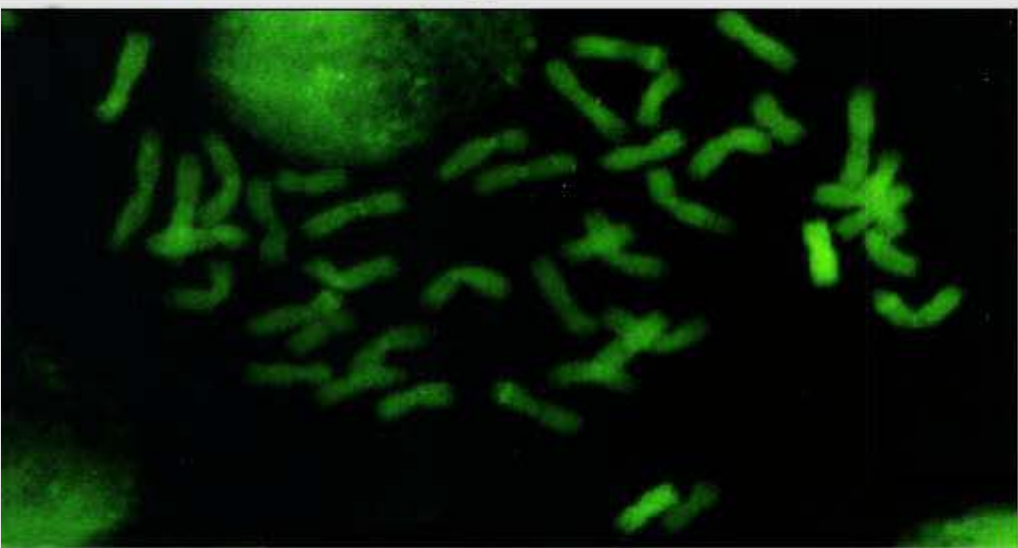
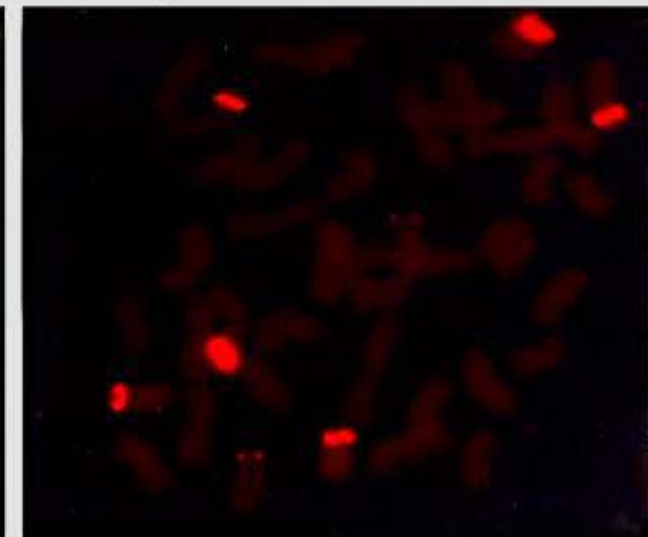
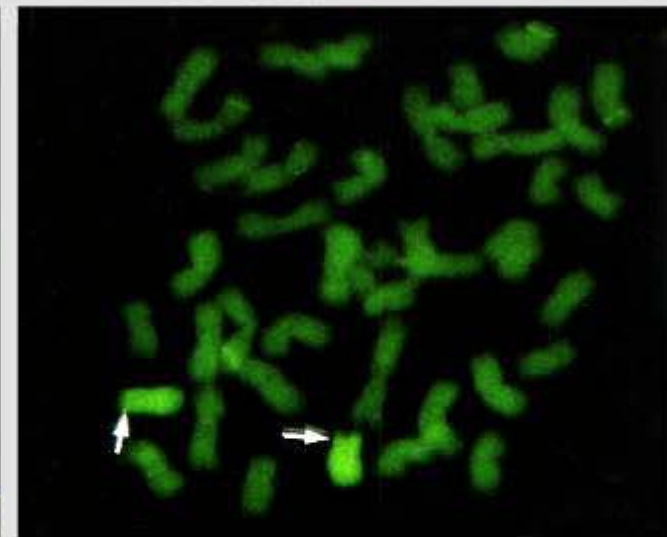
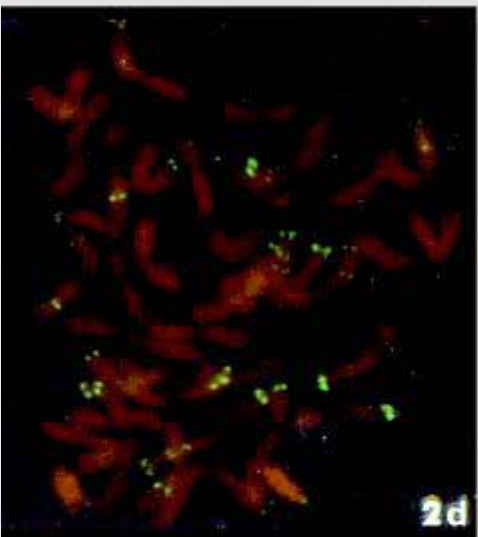
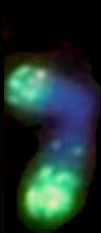
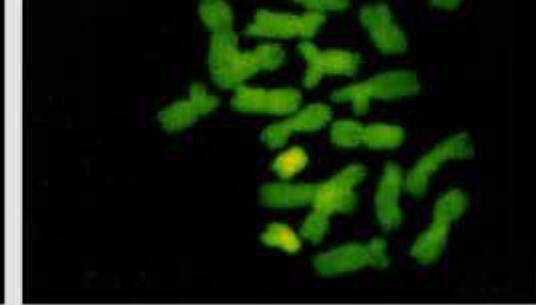
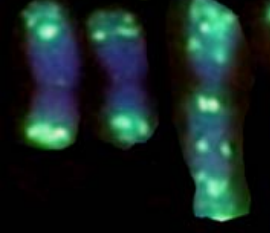
$2n=6x=42$

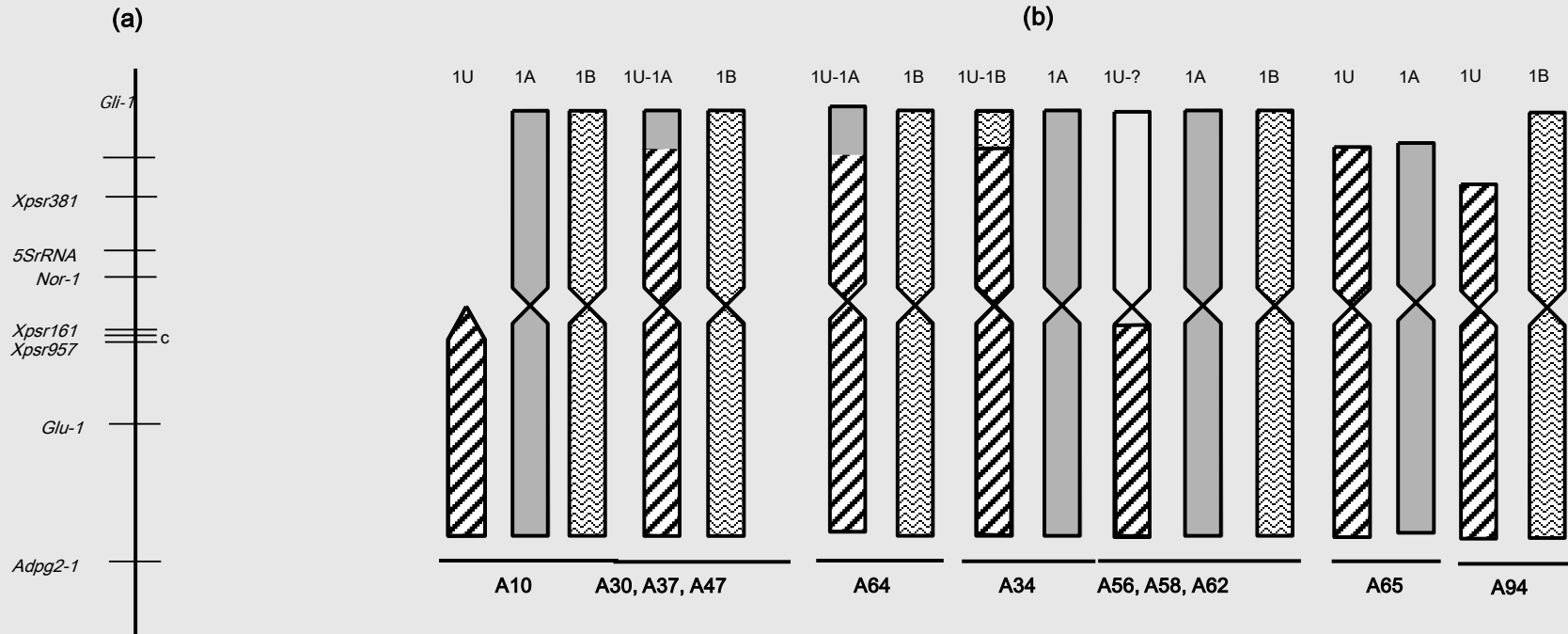
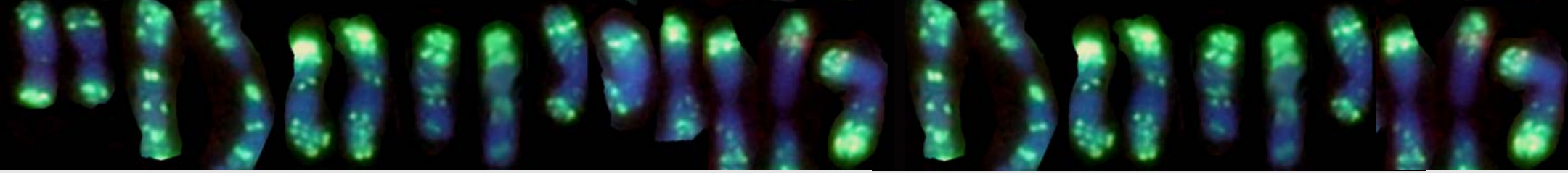
Genomic DNA from
Ae. umbellulata

pSc119.2 repeat



Xana Castilho

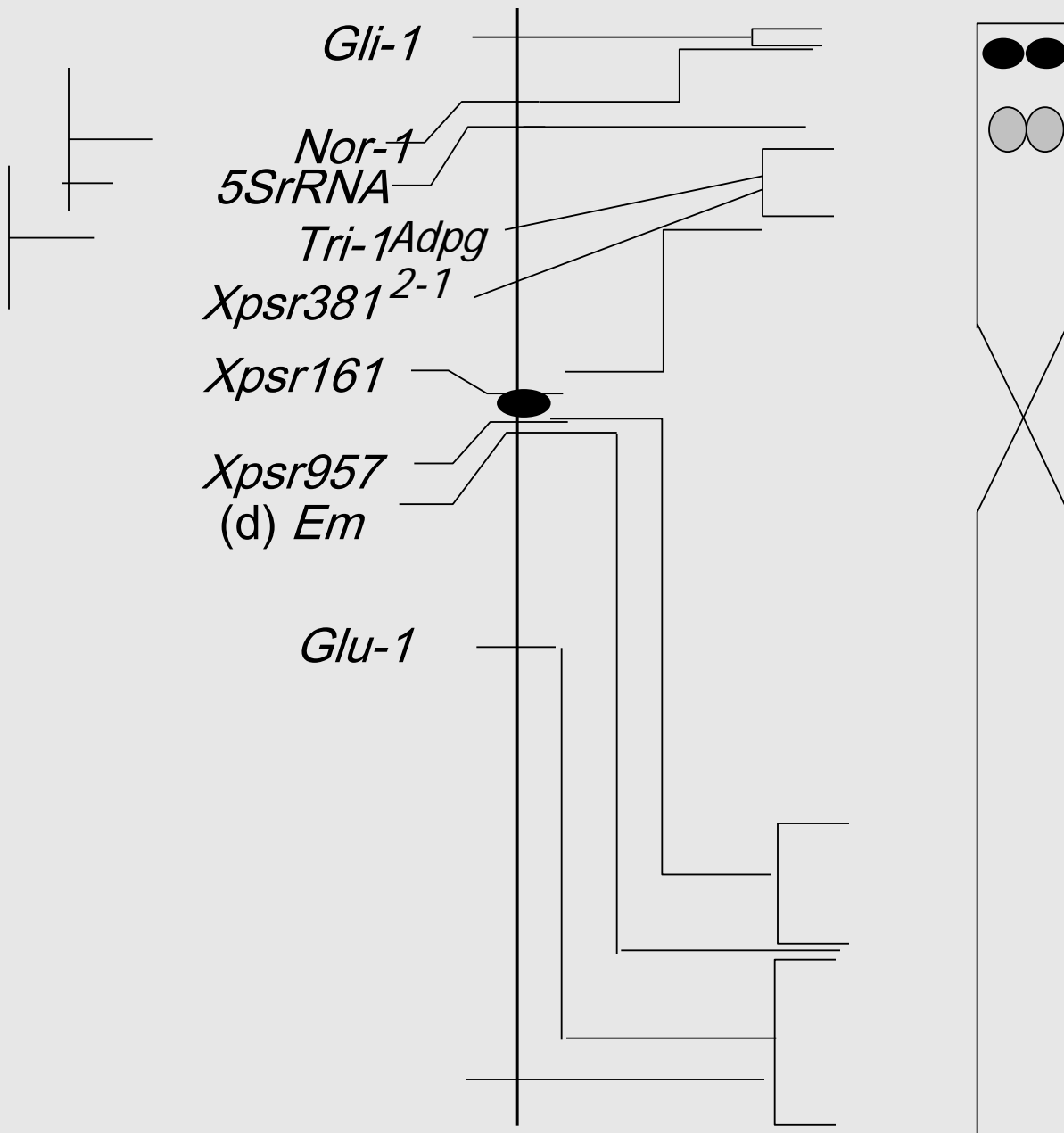




(a) Genetical map of the RFLP probes used. Genetical distances are approximate and based on Gale *et al.*, 1995 for the wheat 1B chromosome.

The order of the *5S rRNA* and *Nor-1* markers is reversed on wheat chromosome 1A .

(b) Physical maps of the wheat-*Ae. umbellulata* lines assigning the RFLP probes on the recombinant chromosome and on the background wheat group 1 chromosomes. all lines carry a pair of 1D chromosome.

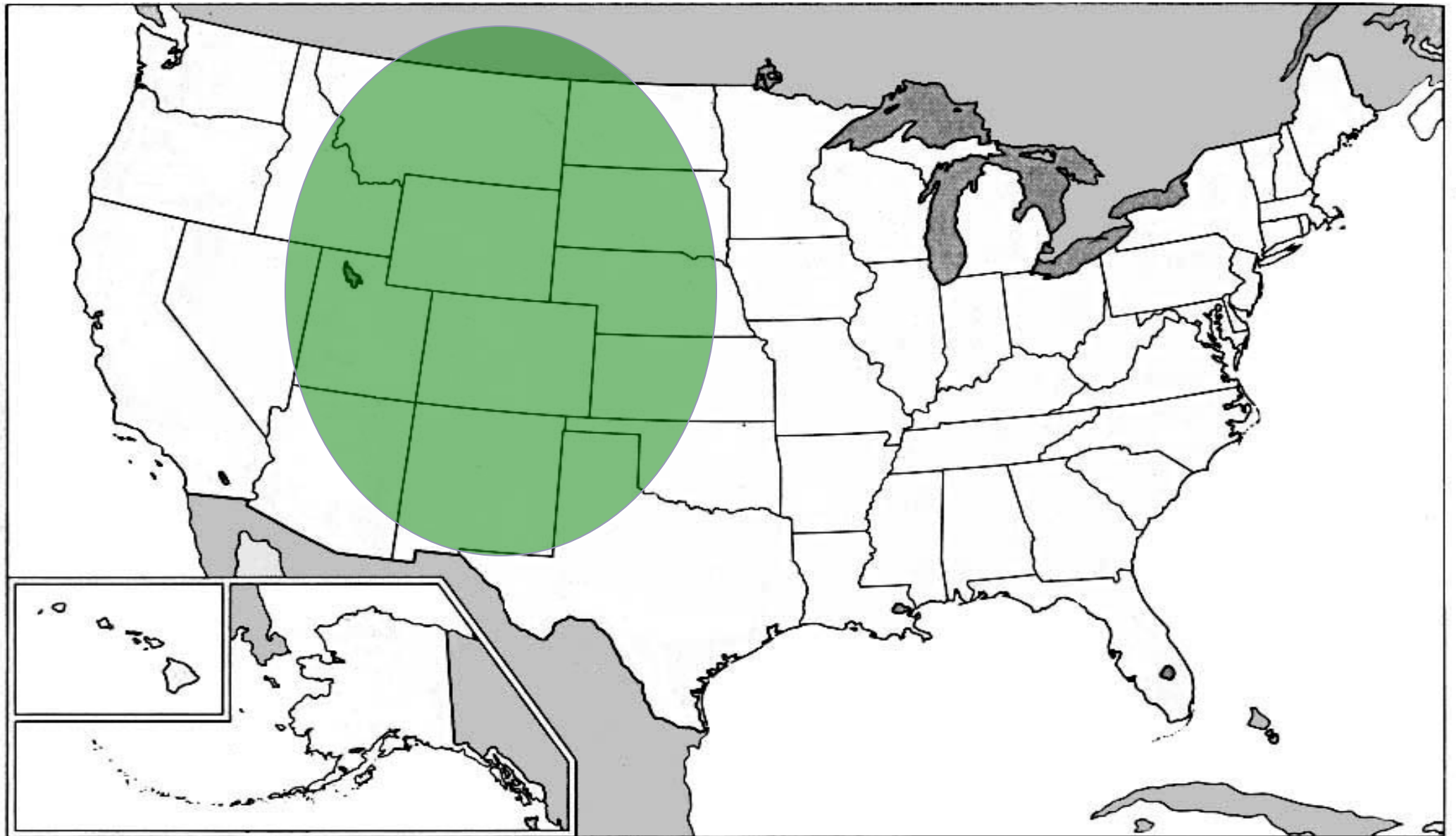


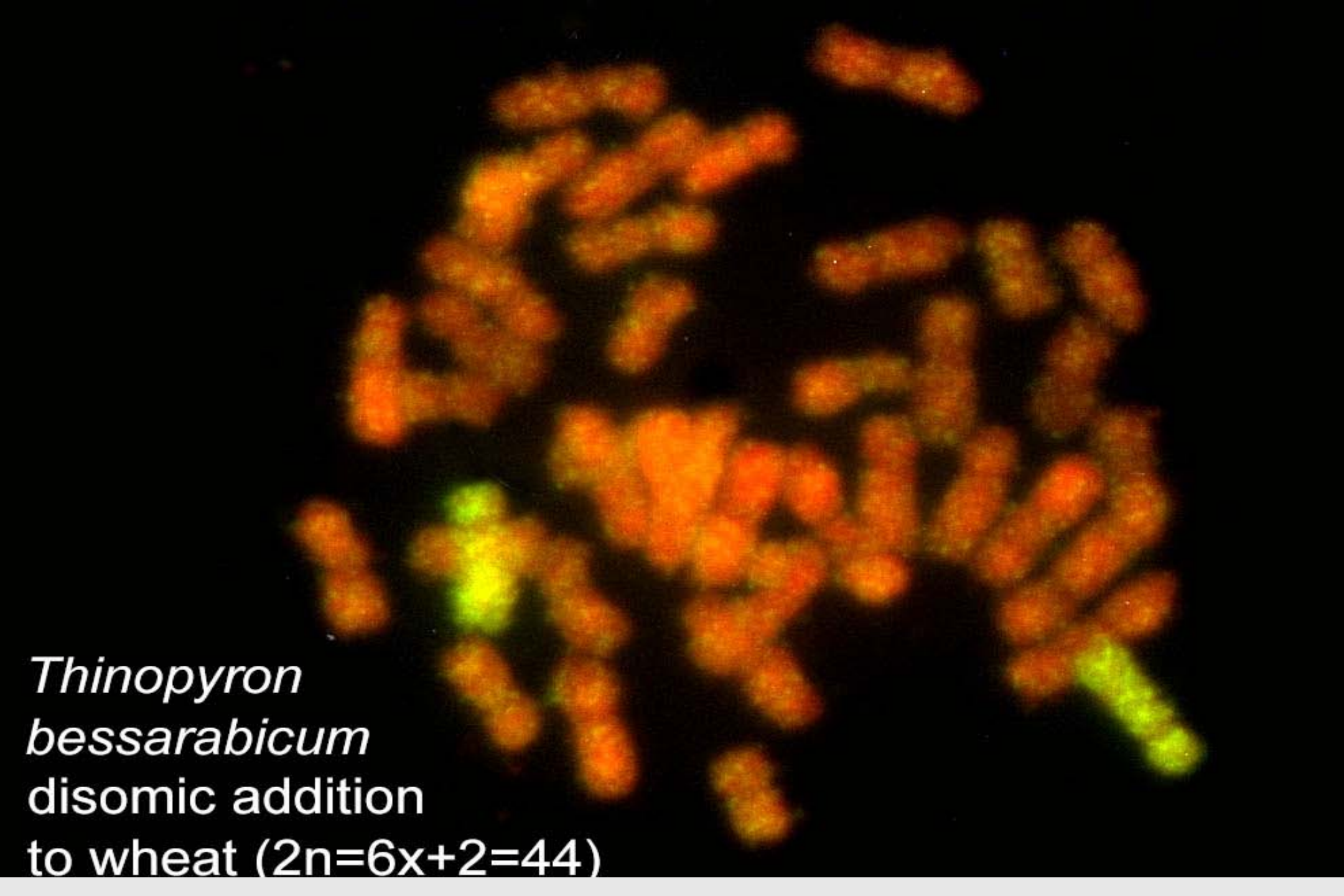
Physical map vs
Genetic map

Genes are often clustered
Genes (and recombination
are often distal

Wheat Streak Mosaic Virus in North America

Bob Graybosch, USDA





*Thinopyron
bessarabicum*
disomic addition
to wheat ($2n=6x+2=44$)

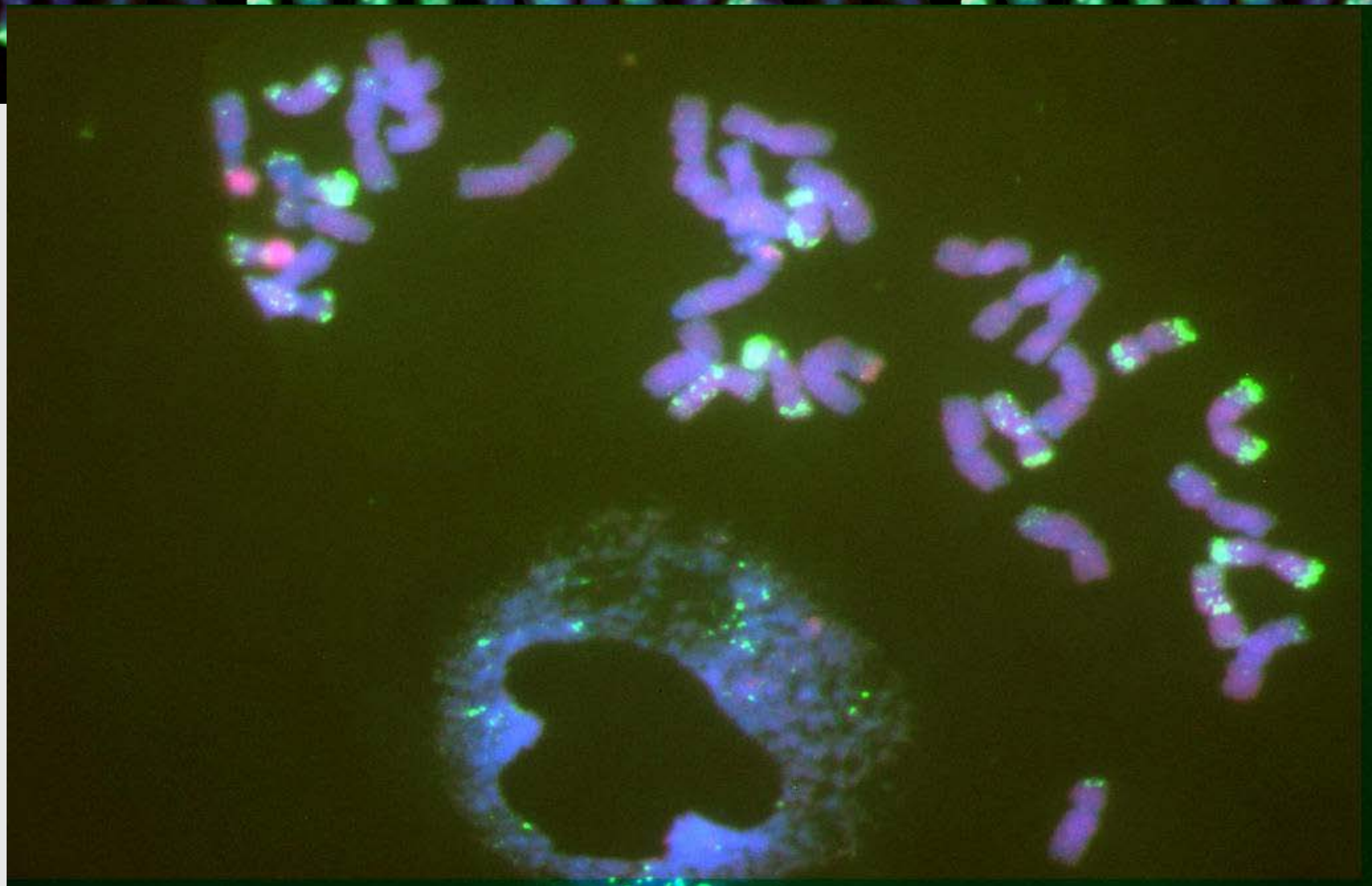
This fluorescence micrograph shows a set of wheat chromosomes with a disomic addition of *Thinopyron bessarabicum*. The wheat chromosomes are stained orange, while the two added chromosomes from *Thinopyron bessarabicum* are stained green. The chromosomes are arranged in a roughly circular pattern, typical of a karyotype preparation.



Wsm-1: only highly effective source of resistance to WSMV



Intermediate wheat-grass, western Nebraska



Registration of 'Mace' Hard Red Winter Wheat

R. A. Graybosch,* C. J. Peterson, P. S. Baenziger, D. D. Baltensperger, L. A. Nelson, Y. Jin, J. Kolmer, B. Seabourn, R. French, G. Hein, T. J. Martin, B. Beecher, T. Schwarzacher, and P. Heslop-Harrison

ABSTRACT

'Mace' (Reg. No. CV-1027, PI 651043) hard red winter wheat (*Triticum aestivum* L.) was developed by the USDA-ARS and the Nebraska Agricultural Experiment Station and released in December 2007. Mace was selected from the cross Yuma//PI 372129/3/CO850034/4/4*Yuma/5/(KS91H184/Arlin S//KS91HW29/3/NE89526). Mace primarily was released for its resistance to *Wheat streak mosaic virus* (WSMV) and adaptation to rainfed and irrigated wheat production systems in Nebraska and adjacent areas in the northern Great Plains. Mace was derived from a head selection made from a heterogeneous, in terms of field resistance to WSMV, F₅ line. Resistance to WSMV is conditioned by the *Wsm-1* gene, located on an introgressed chromosome arm from *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey [*Agropyron intermedium* (Horst.) Beauv.] present as a 4DL.4AgS chromosomal translocation. Mace was tested under the experimental designation N02Y5117.

Abbreviations: NRPN, Northern Regional Performance Nursery; PCR, polymerase chain reaction; WSBMV, *Wheat soilborne mosaic virus*; WSMV, *Wheat streak mosaic virus*.

Published in the Journal of Plant Registrations 3:51–56 (2009).

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such line, subsequently named 'Mace' (Reg. No. CV-1027, PI 651043), was deemed suitable for cultivar release. Mace is a hard red winter wheat cultivar developed cooperatively by the USDA-ARS and the Nebraska Agricultural Experiment Station and released in 2007 by the developing institutions. Mace was released primarily for its field resistance to *Wheat streak mosaic virus* (WSMV) and adaptation to rainfed and irrigated wheat production systems in Nebraska and adjacent areas in the northern Great Plains. Resistance to WSMV is conditioned by the *Wsm-1* gene (Seifers et al., 1995), situated on an introgressed chromosome arm from





Wild banana species:

Musa acuminata – A genome

genome

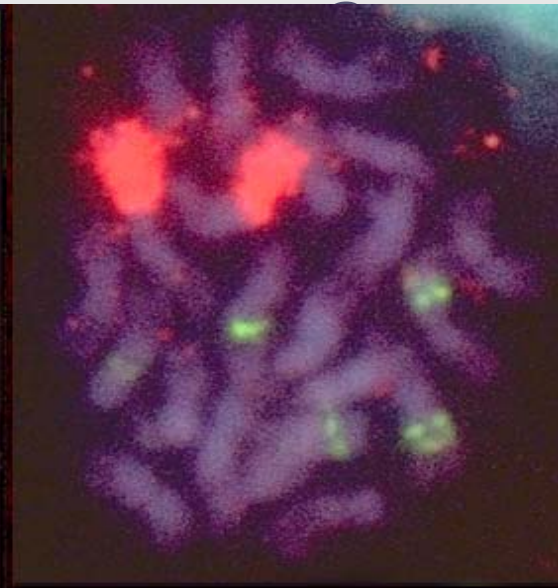
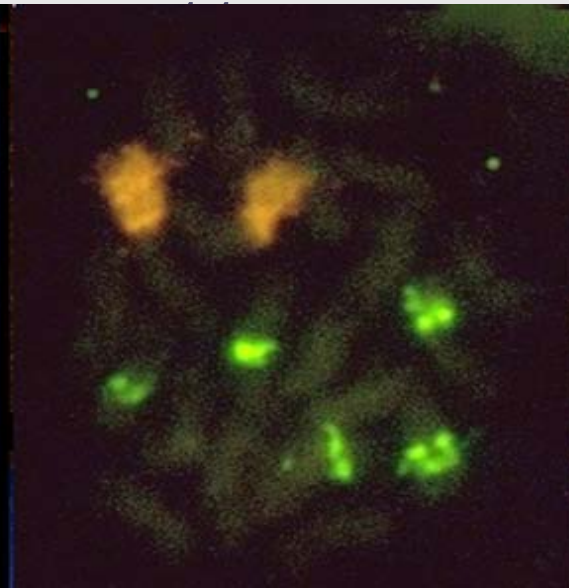
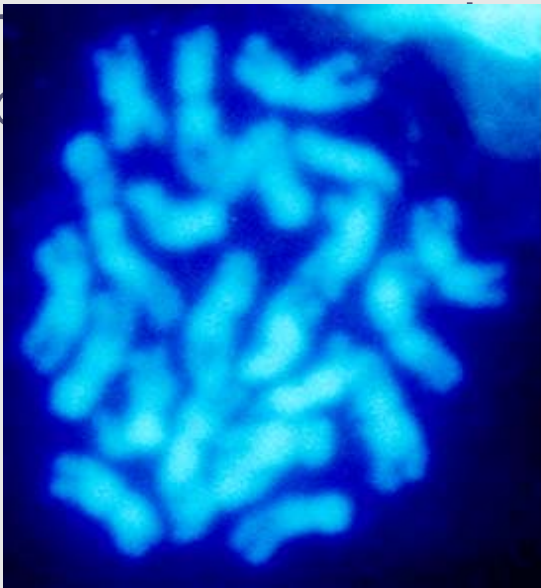
Basic ch

550 Mbp

Most cu

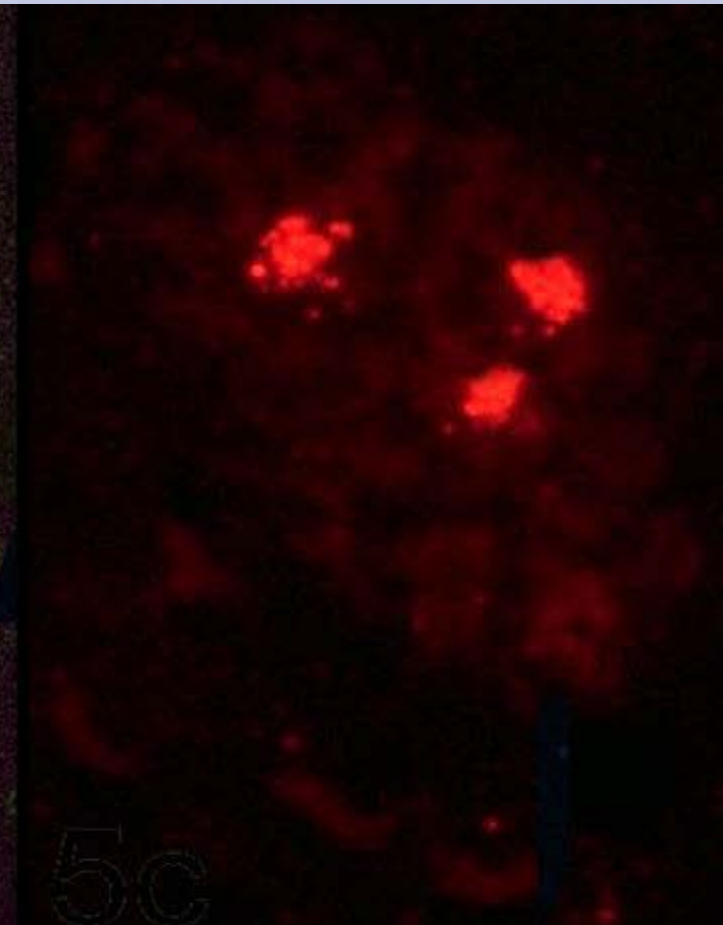
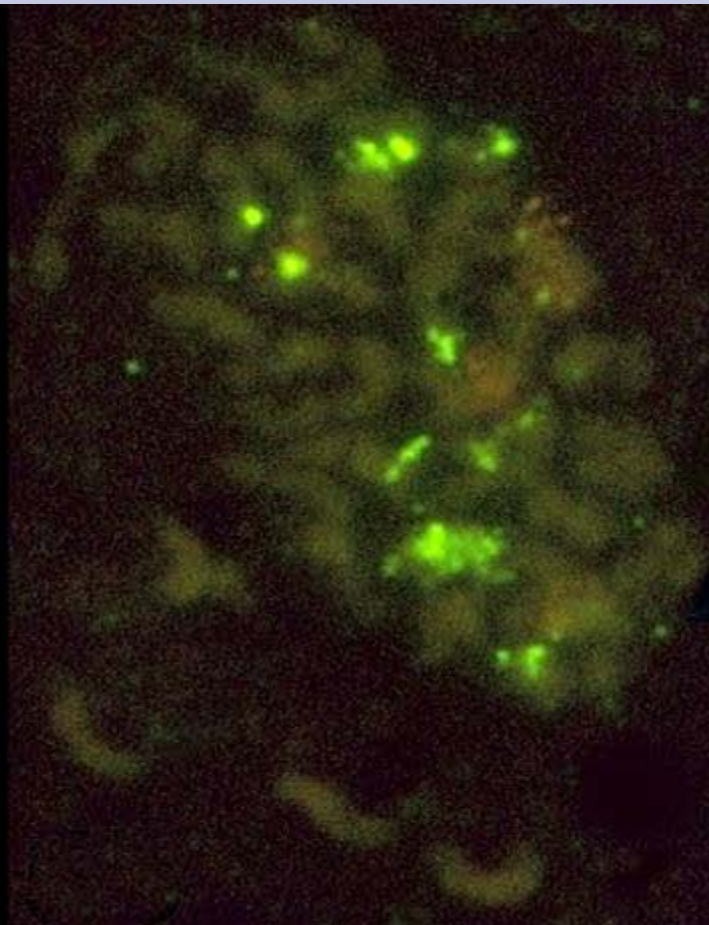
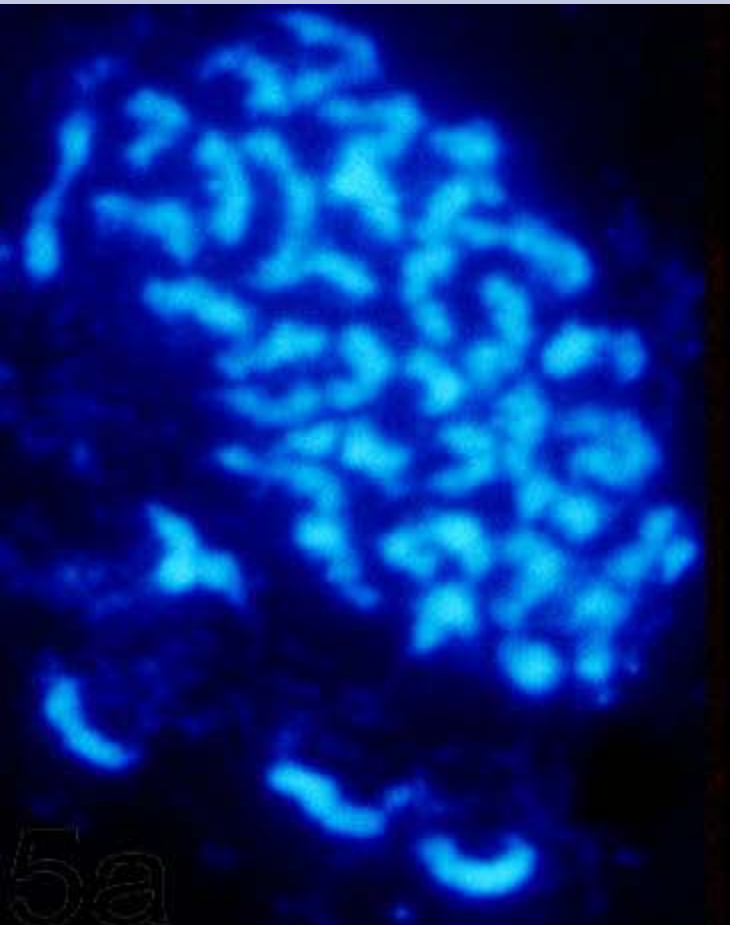
Musa balbisiana – B

genome size c.



Cavendish : the most common
dessert banana cultivar

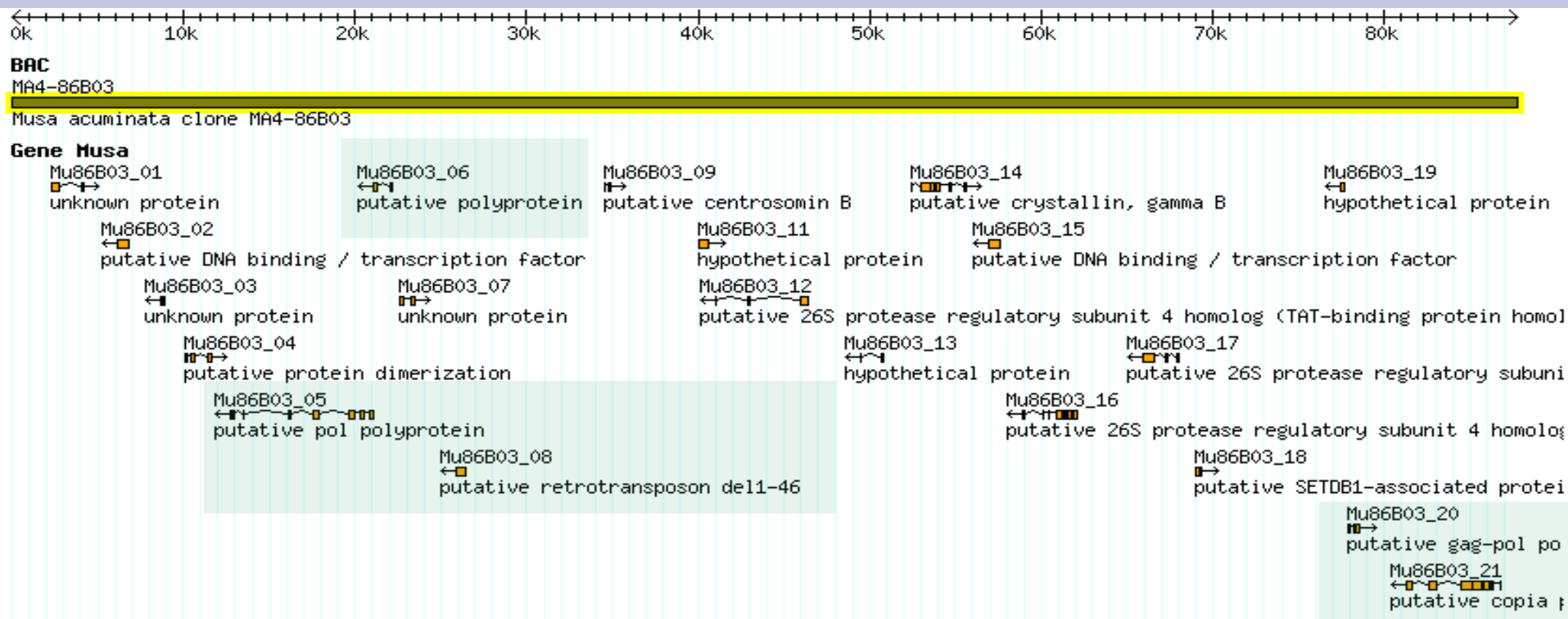
$2n=3x=33$; AAA genomes



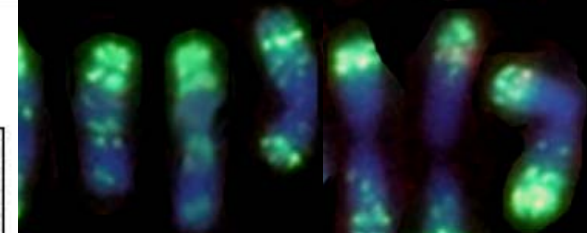
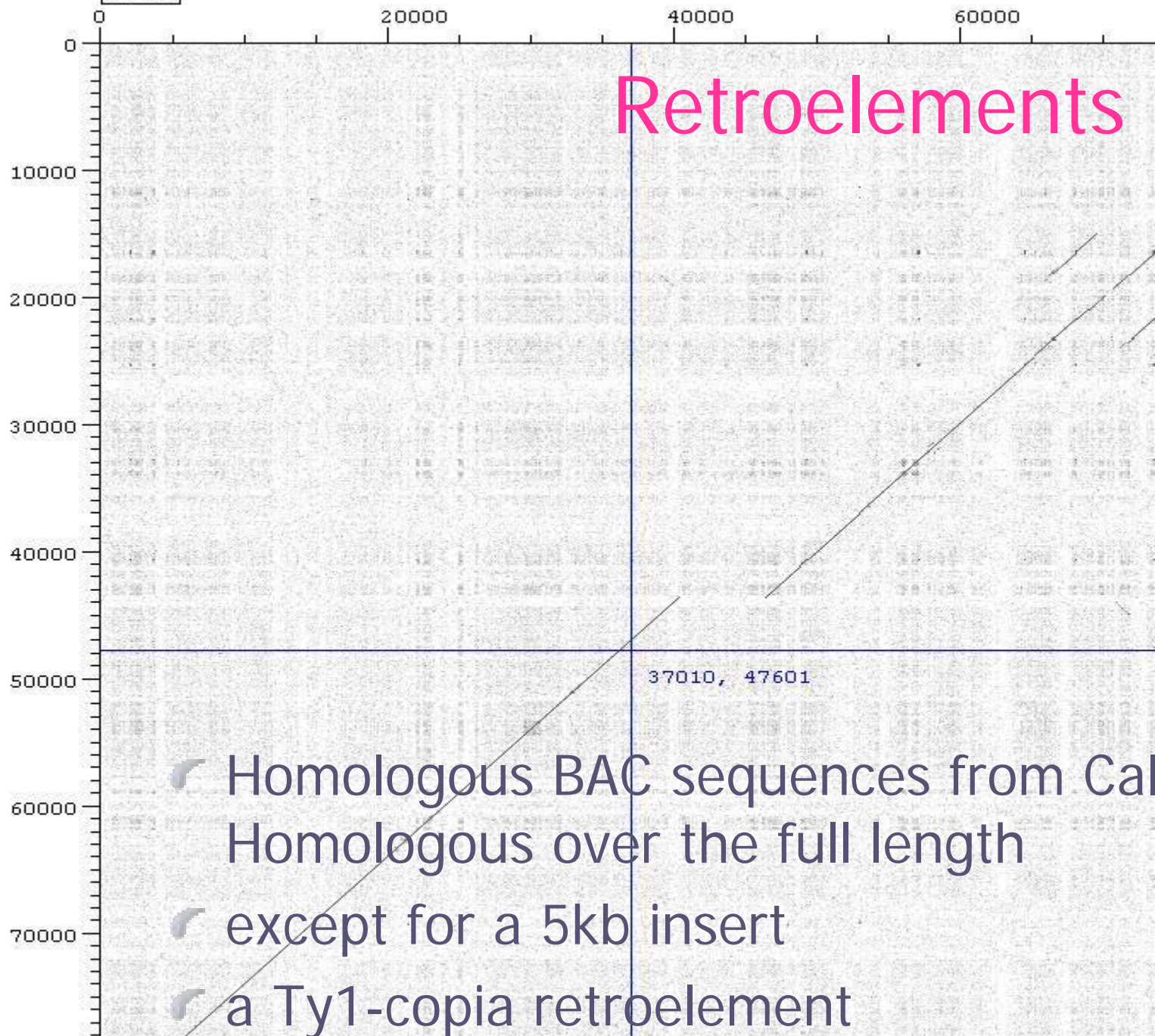
Retroelements

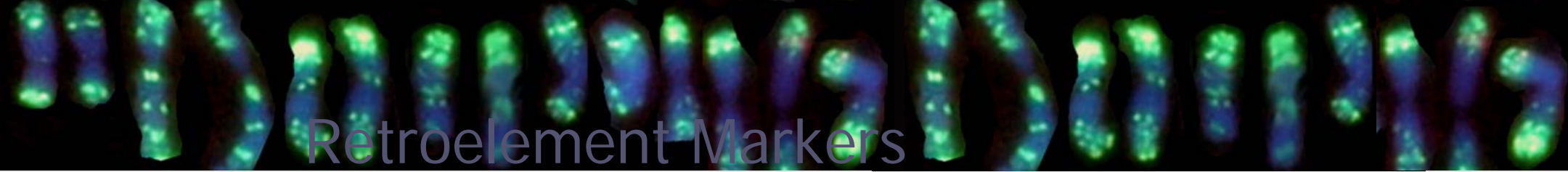
Sequences which amplify through an RNA intermediate

30% to 50% of all the DNA!



About



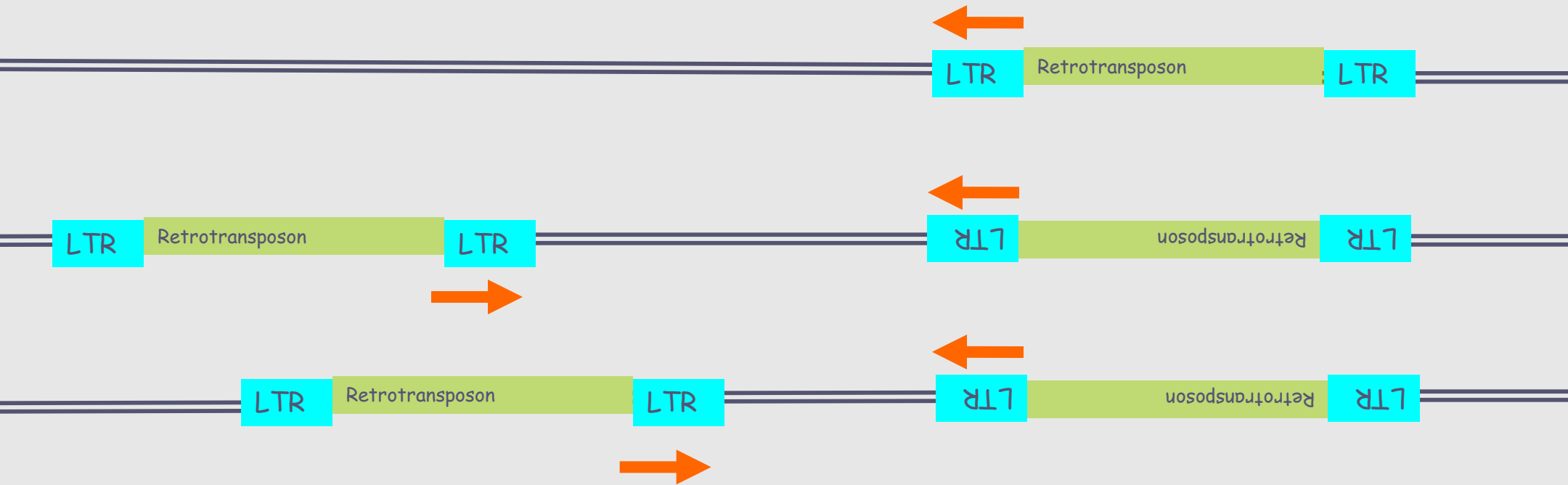


Retroelement Markers

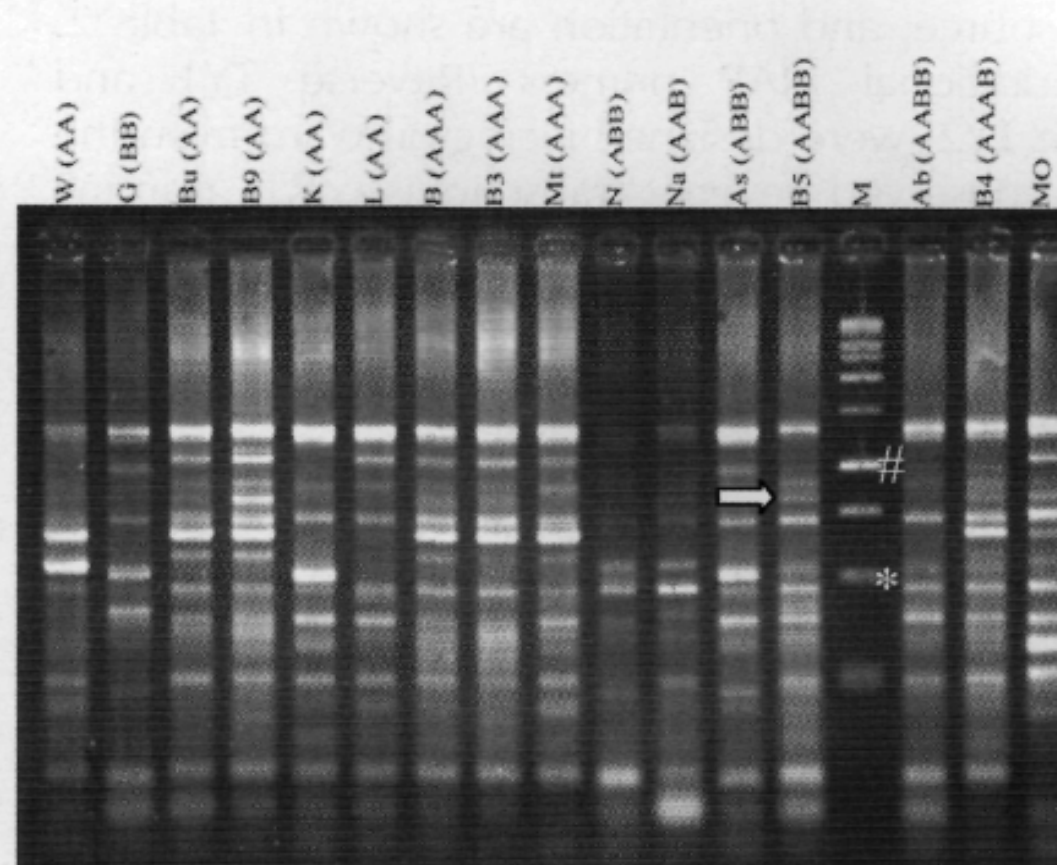
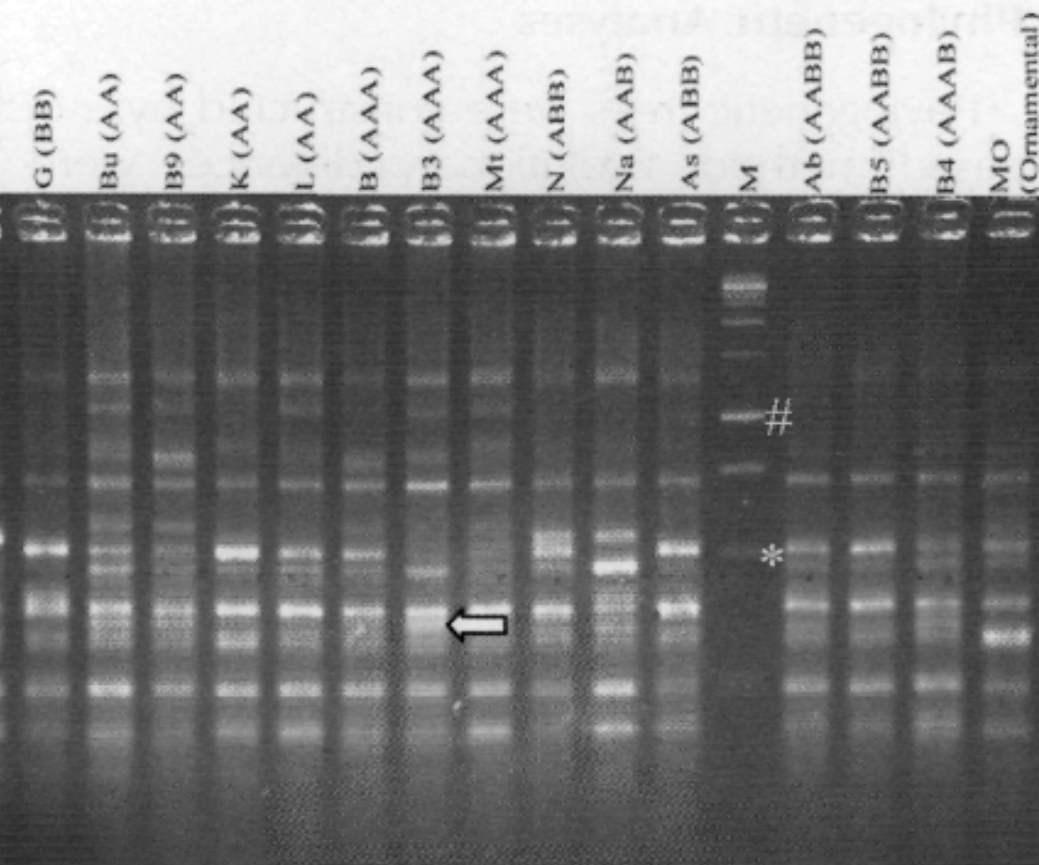
Insertion



IRAP - InterRetroelement PCR

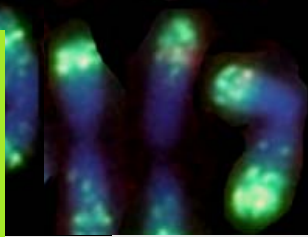


IRAP diversity in Musa

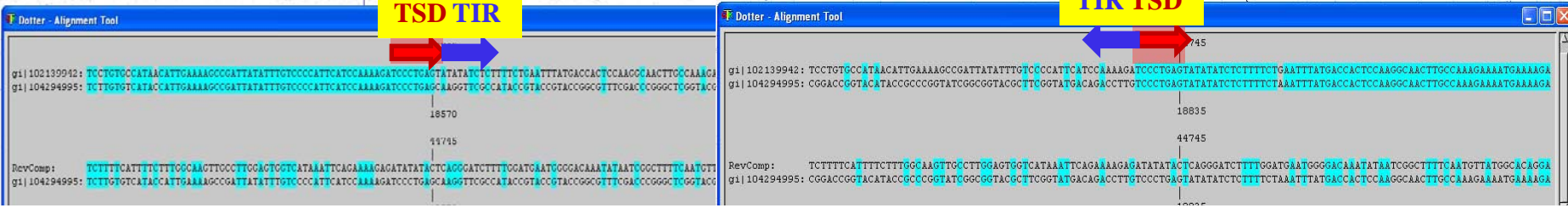


Teo, Tan, Ho, Faridah, Othman, HH, Kalendar, Schulman 2005 *J Plant Biol*

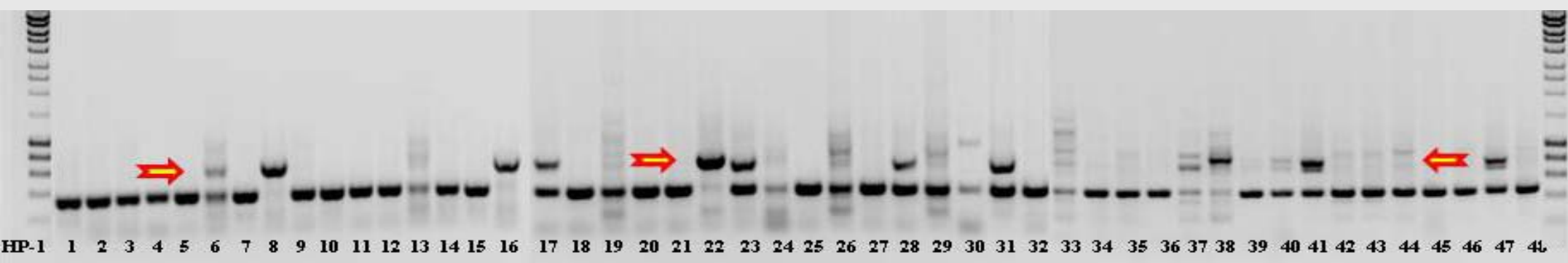
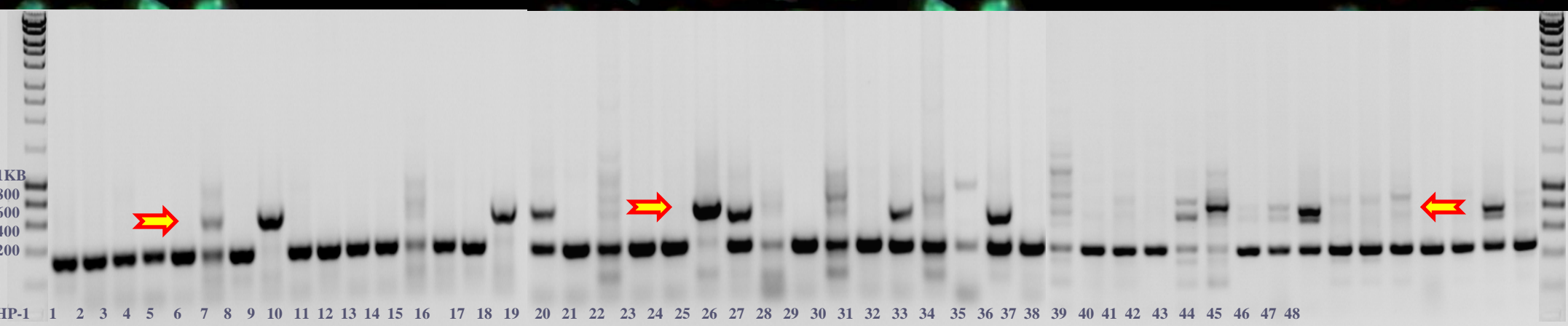
Noir, Teo, Schwerzger, HH 2006 *Euphytica*



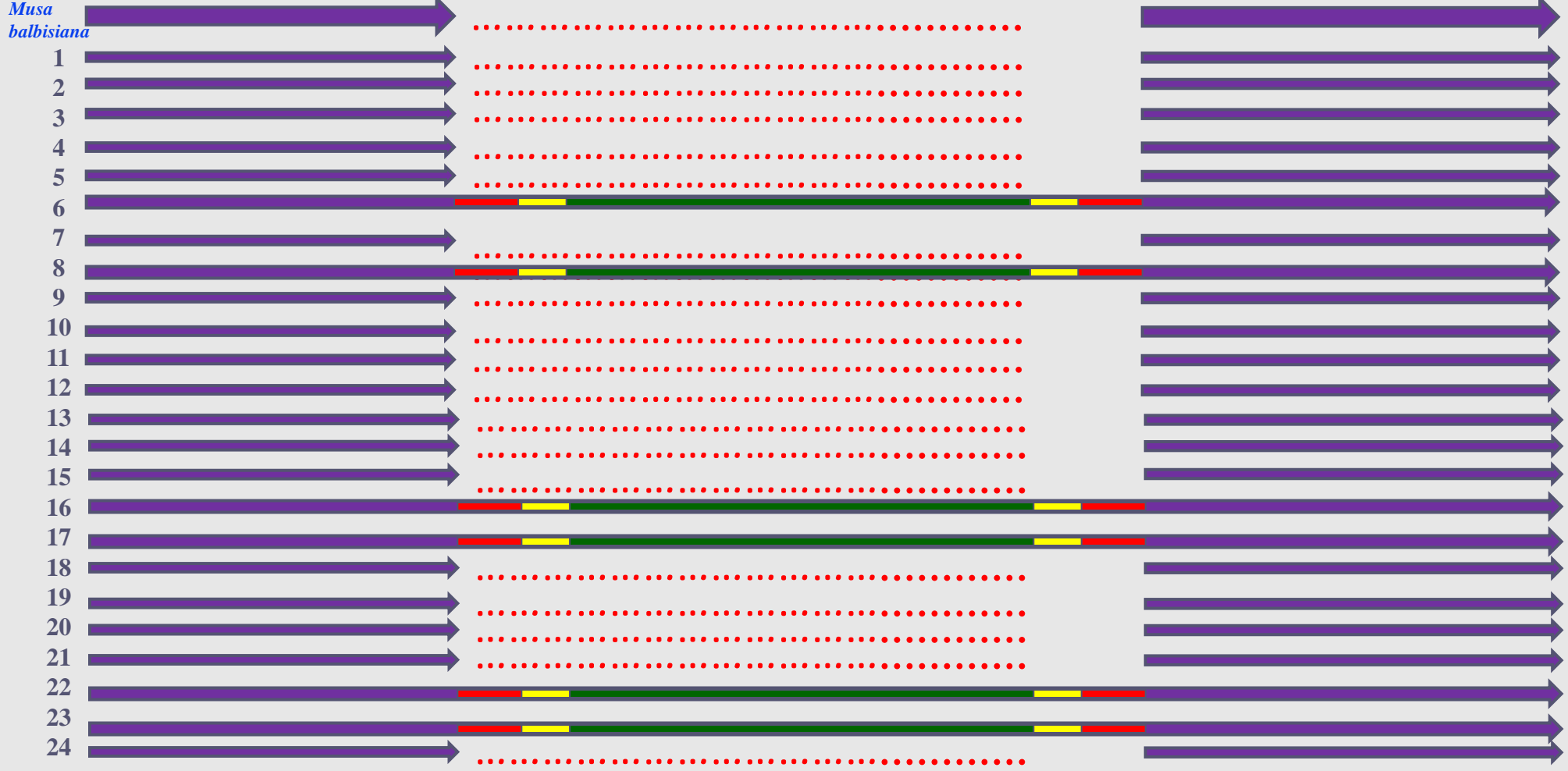
ACCCACCTGGCTCTTGTGTCATACCATTGAAAAGCCGATTATATTTGTCCCCATTCATCCAAAAGA **TCCCTGA**
GCAAGGTCTGCCATAACCGTACCGTACCGGCGTTTCGAC**CCGG**GCTCGGTACGGTA**CCGG**TGTAC**CCGG**GCAG
TACATCAGGGTGTACCGAATGGTACACCCTGATGTACCGAACAATTTTATACTTTTTCATACTGTAGCAGTGCT
ACAGTATAATACTGTAGCACTGTAGCGGTATCGGGCGGTCCGCGTA**CCGG**TAACCTGTCCGA**CCGG**TACATAC
CGC**CCGG**TATCGGCGGTACGCTTCGGTATGACAGACCTTGT**TCCCTGAG**TATATATCTCTTTTCTAAATTTATG
ACCACTCCAAGGCAACTTGCCAAAGAAAATGAAAAGAAGAAAAAATTAGGGGAATGAAGATTCTCCACA
ATTCTTATTCTTTGATTTGAGATAATAATGTCCATAGTAAAACATATCTTATGATCATCATTGCTGATTAATCA
AAATACCTGATTCTATAGTCTCAAGCTTTAGTGGTCAAAACACATTCCG



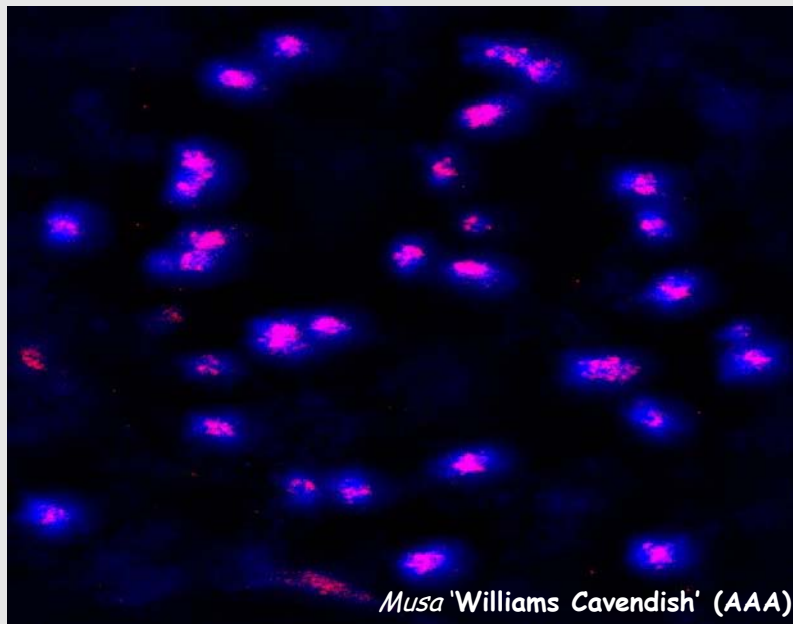
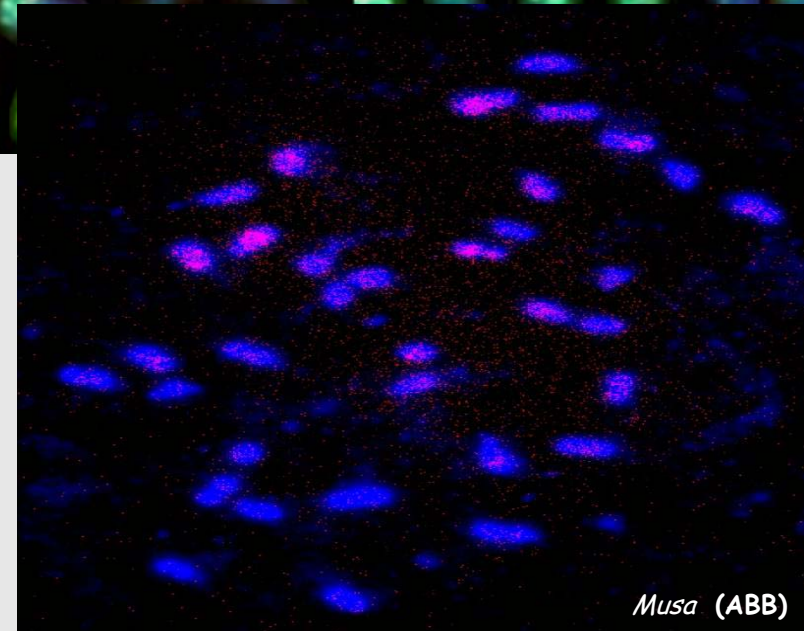
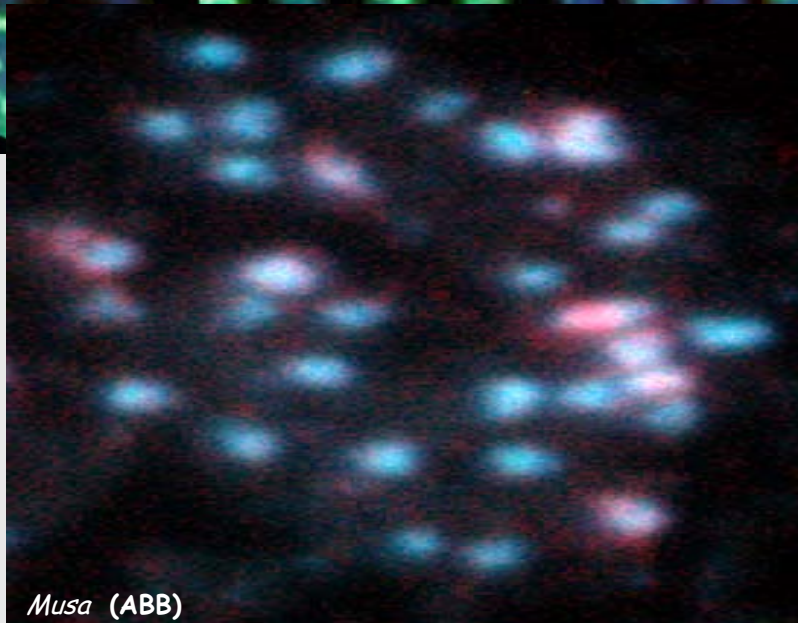
hAT1 in *Musa acuminata*
F and R primers indicated by blue arrows in sequence



hAT1 insertion sites in *Musa* diversity collection
 hAT486F and hAT037R
 Top bands (560-bp) amplified hAT element and lower bands amplifying the



The hAT1 insertion sites in Musa with hAT486F and hAT037R in: 6) acuminata, 8) acuminata, 16) acuminata, 17) AAB, 22) acuminata, 23) AB cv.



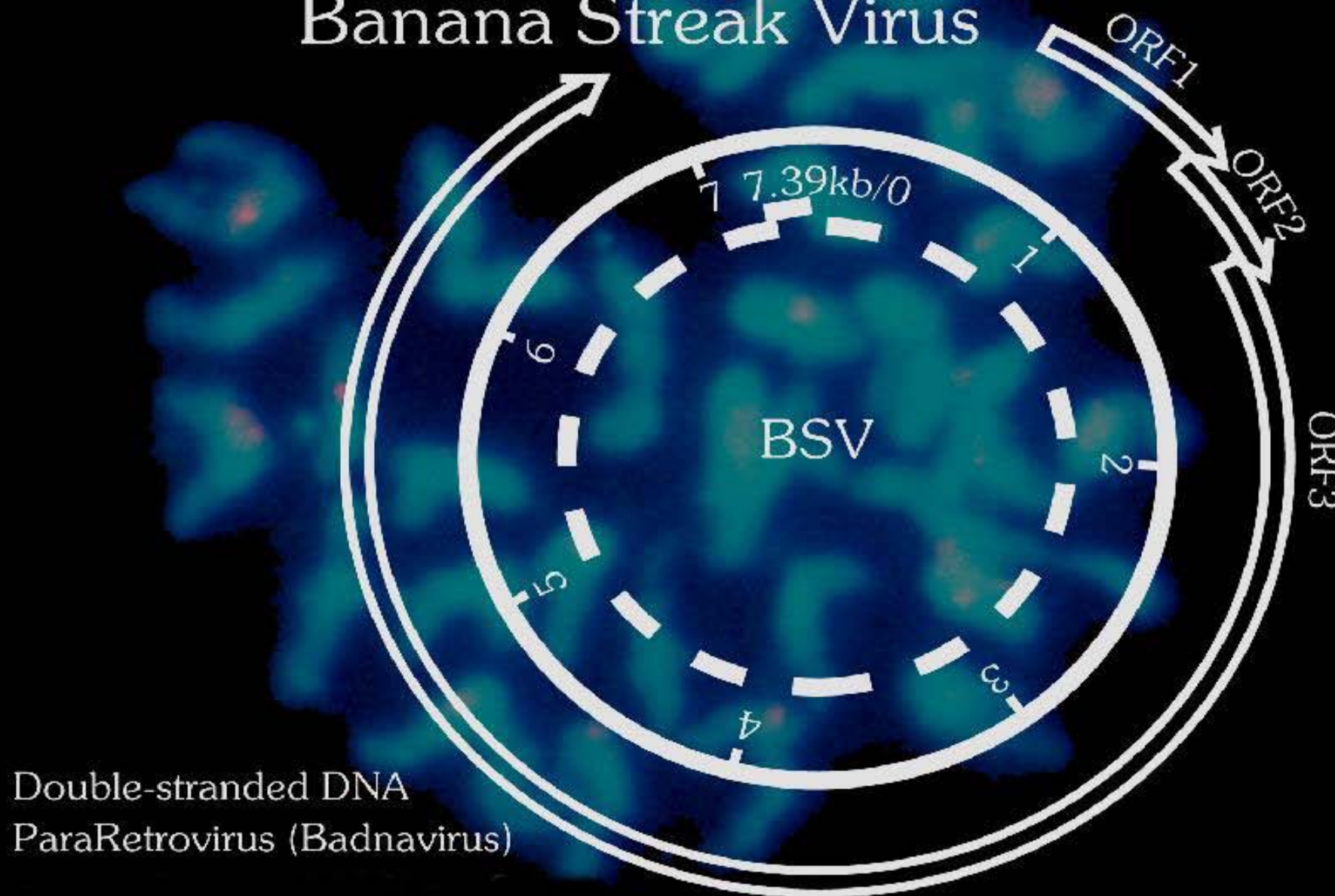
A-genome specific hAT in
three *Musa* hybrids
($2n=3x=33$)

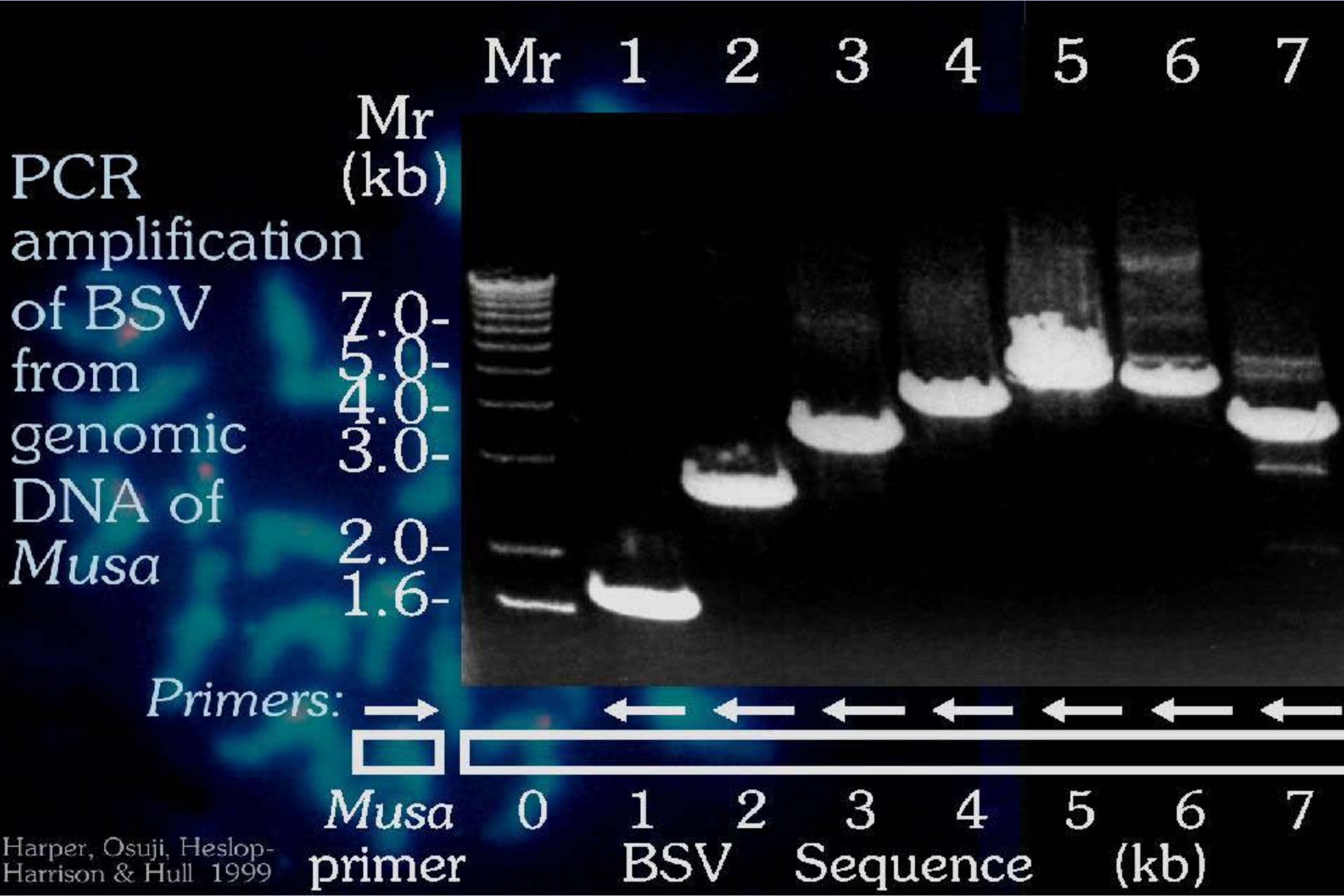
Banana Streak ParaRetrovirus (BSV)

- 👉 Double stranded DNA is infective
- 👉 Insect vector
- 👉 Unexpected epidemiology
 - Appearance after cold or tissue culture

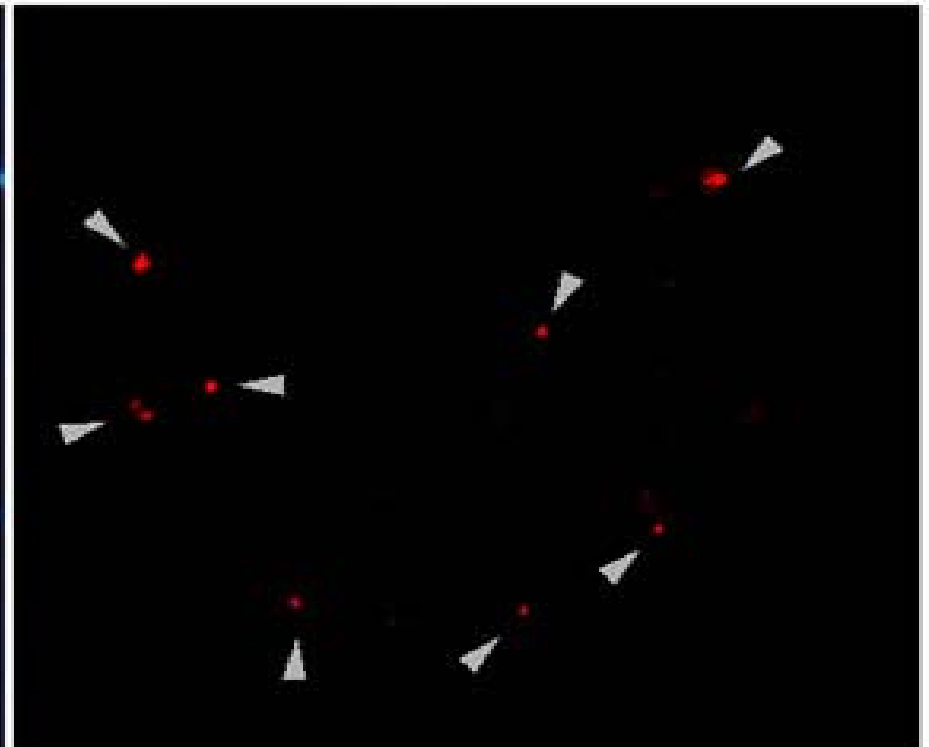
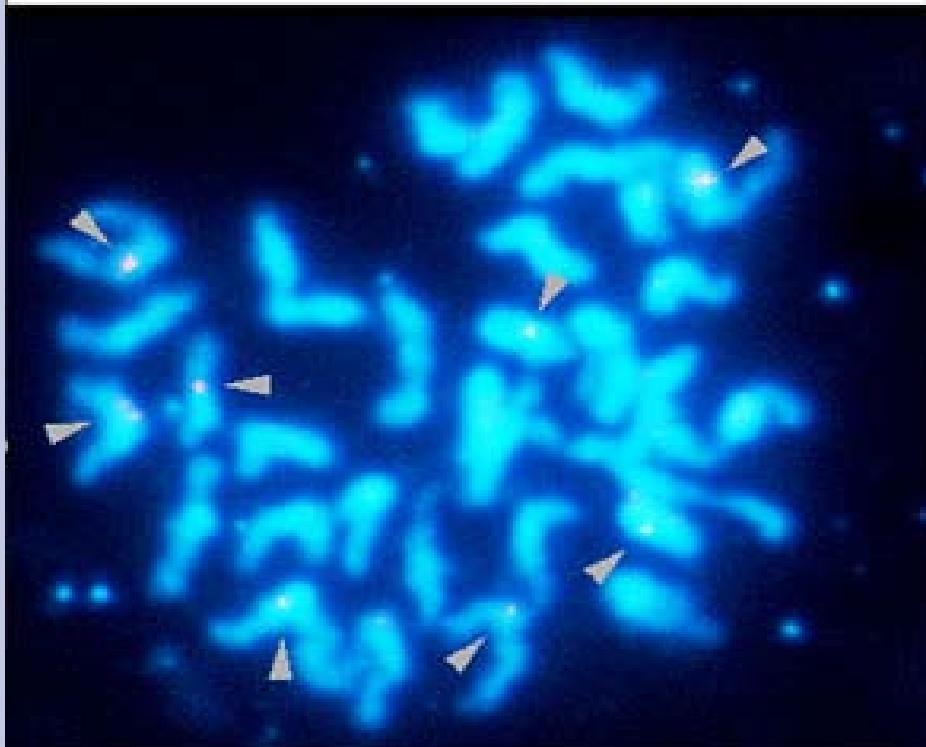
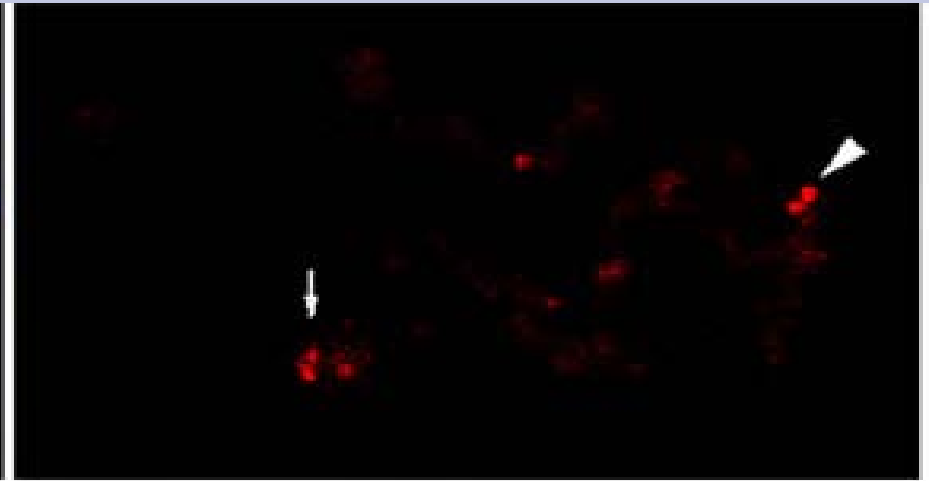
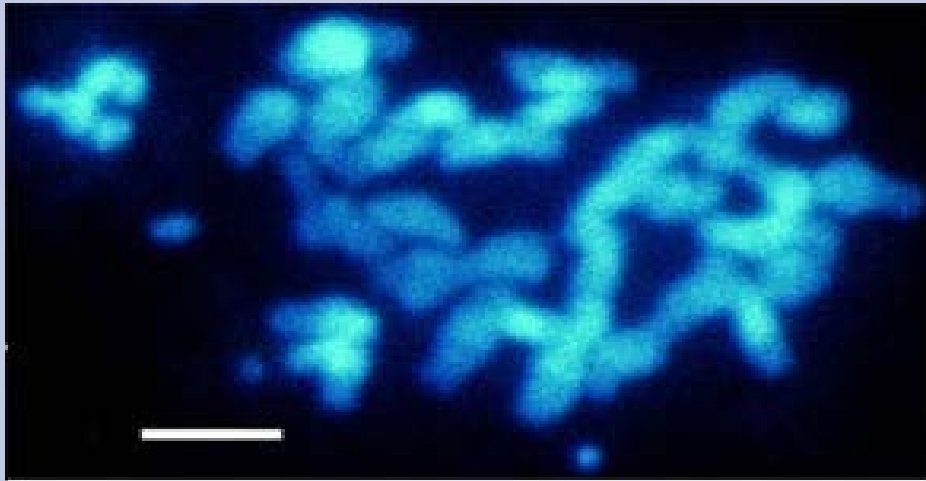
Glyn Harper & Roger Hull

Banana Streak Virus

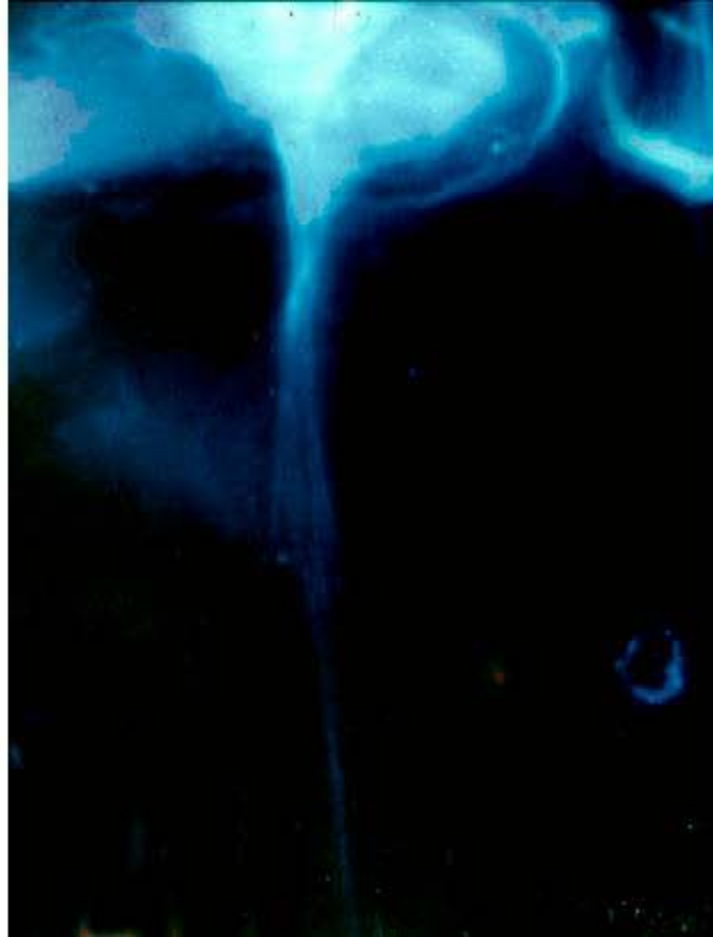
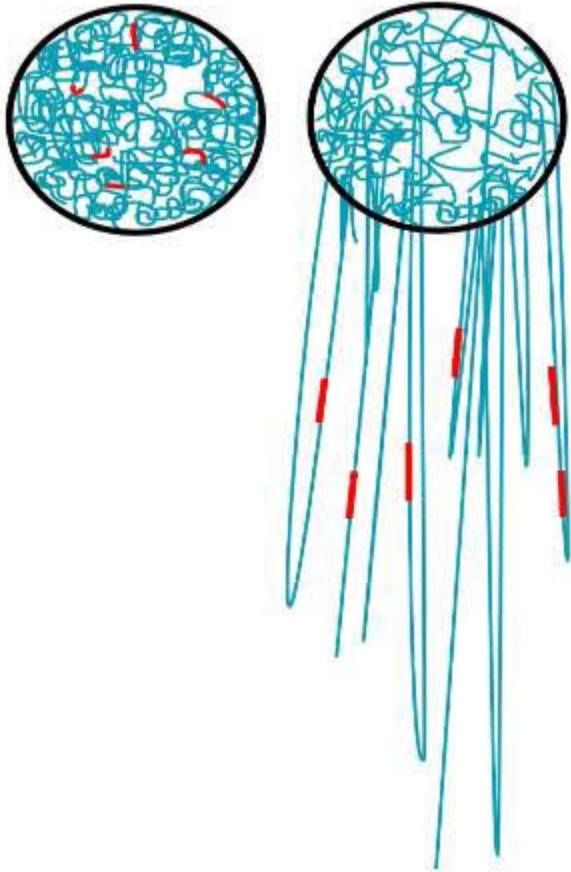




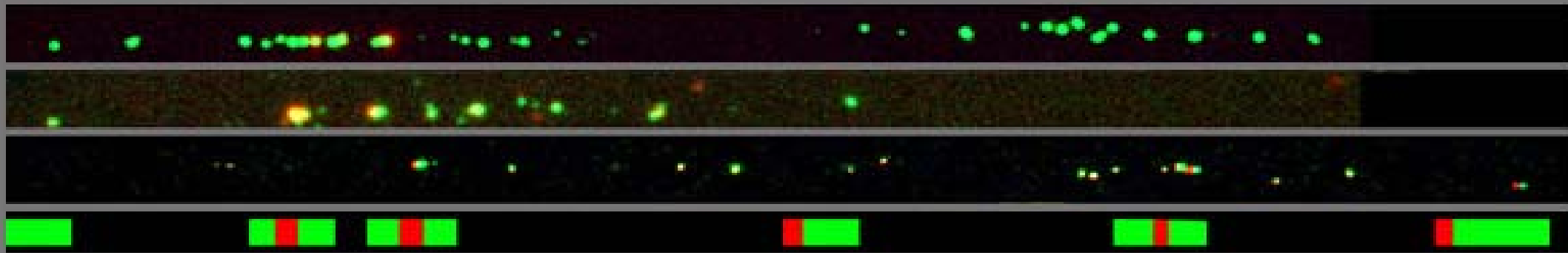
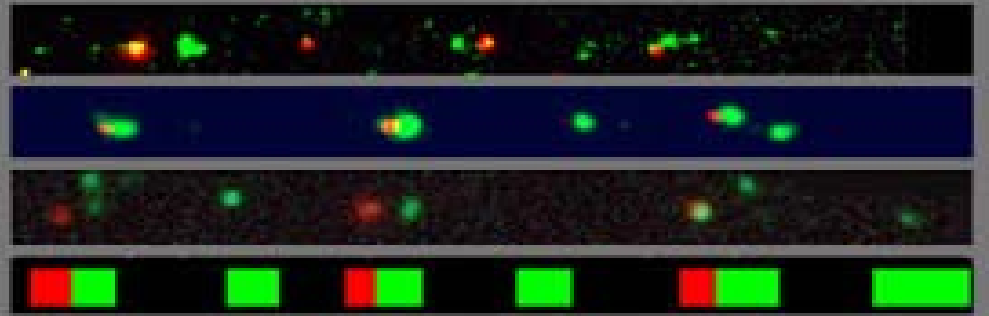
Nuclear Copies of BSV in Banana



DNA Fibre Hybridization



Nuclear Copies of BSV in Banana



Oil palm: *Elaeis guineensis*



Malaysian Palm Oil Board
Alex Vershinin, Sybille Kubis,
Maria Madon, Xana Castilho,
Trude Schwarzacher



Deli Dura (D)



DxP



Pisifera (P)



Epigenetics

Phenotype appears 5 years after tissue culture





Modulation of Methylation

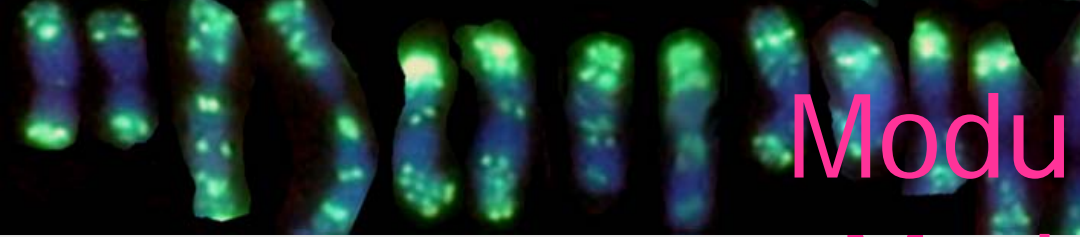
McrBC

unusual restriction enzyme cutting between methylated cytosine (^mC) sites

Cuts ^mC NNNNNNNNNNNN ^mC

Leaves C NNNNNNNNNNNN C

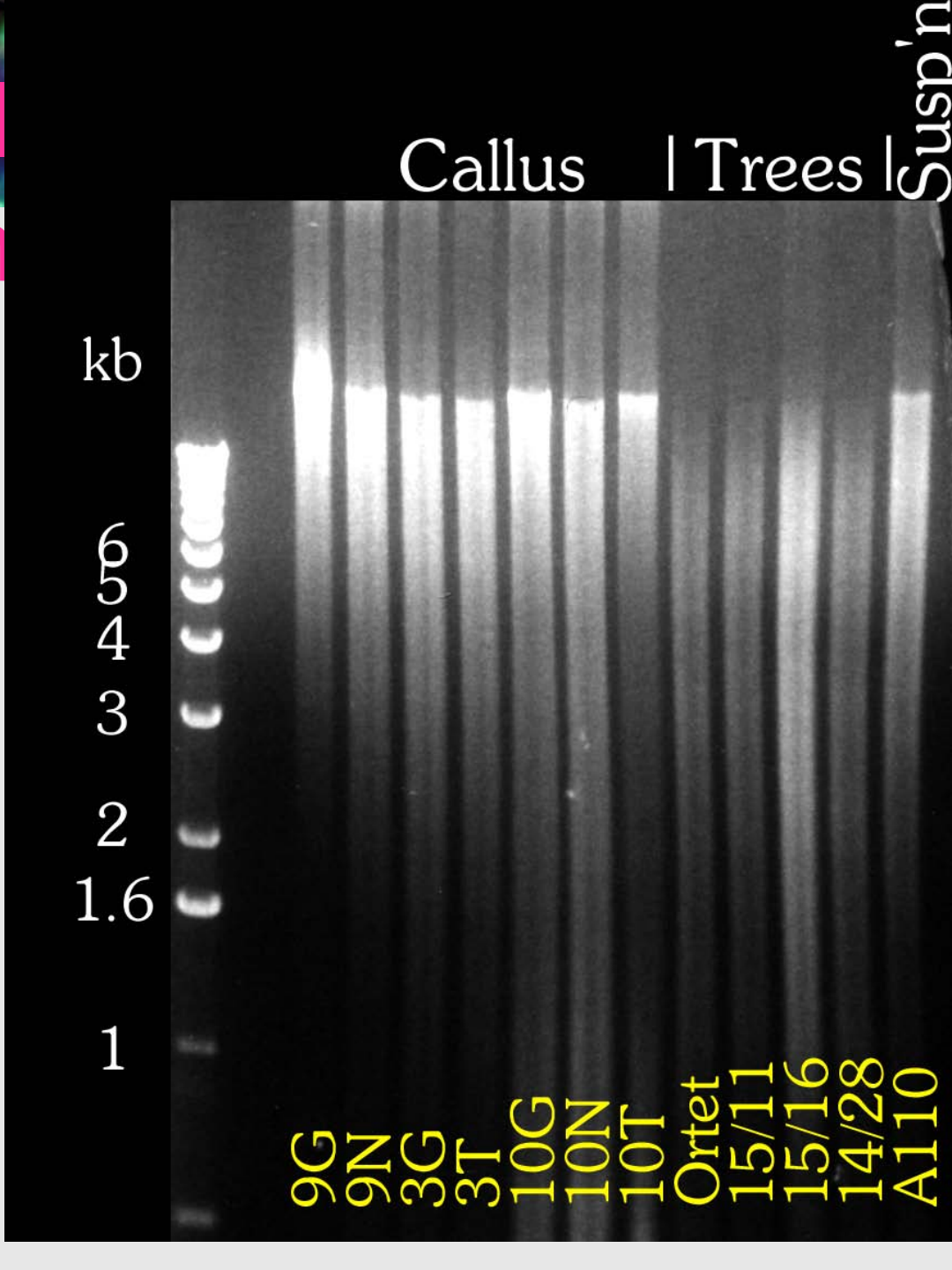
N typically 20 to 40 bases

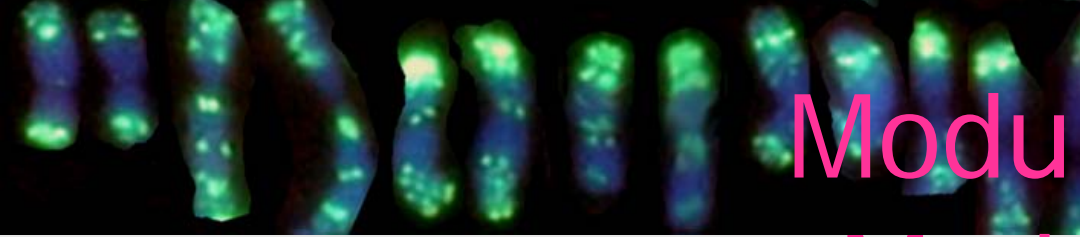


Modul Meth

- McrBC - shows substantial reduction in methylation in tissue culture lines
- Cuts methylated DNA

Kubis, Castilho, Vershinin, HH 2003



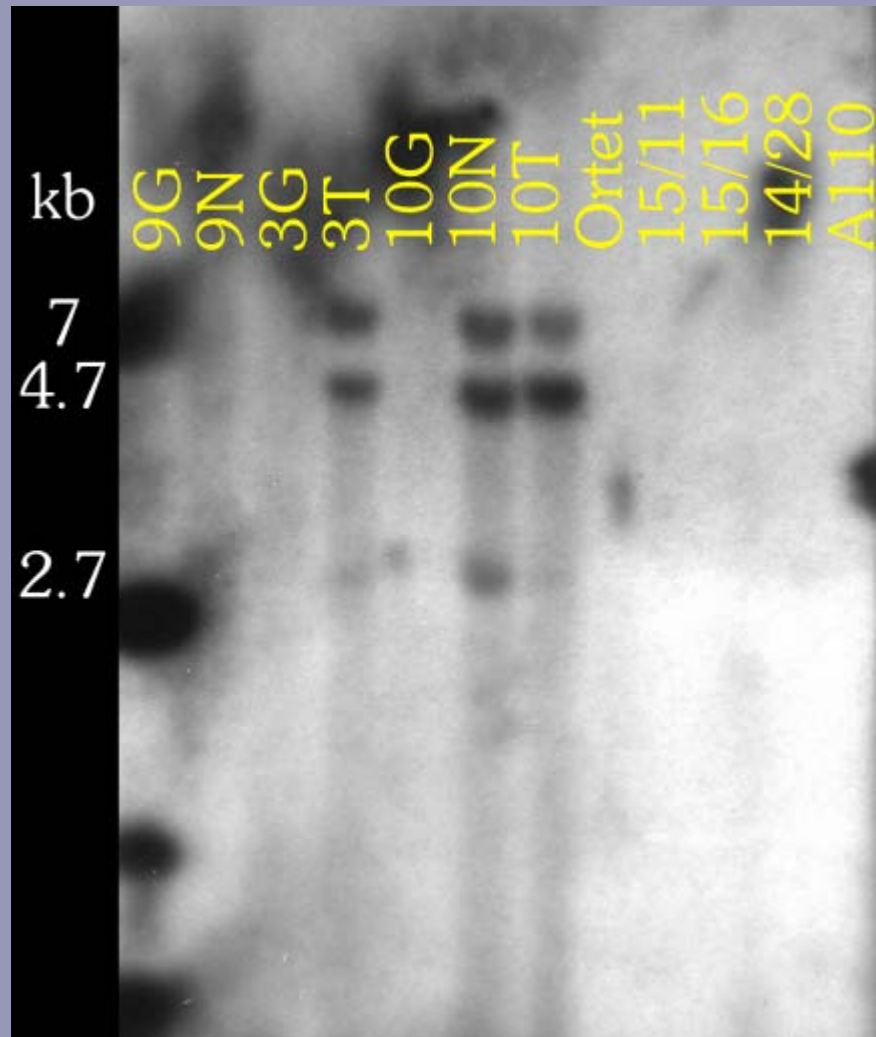


Modul Meth

- ☛ McrBC digests probed with *gypsy* clones
- ☛ present only in N and T lines
- ☛ Similar with *copia* probe

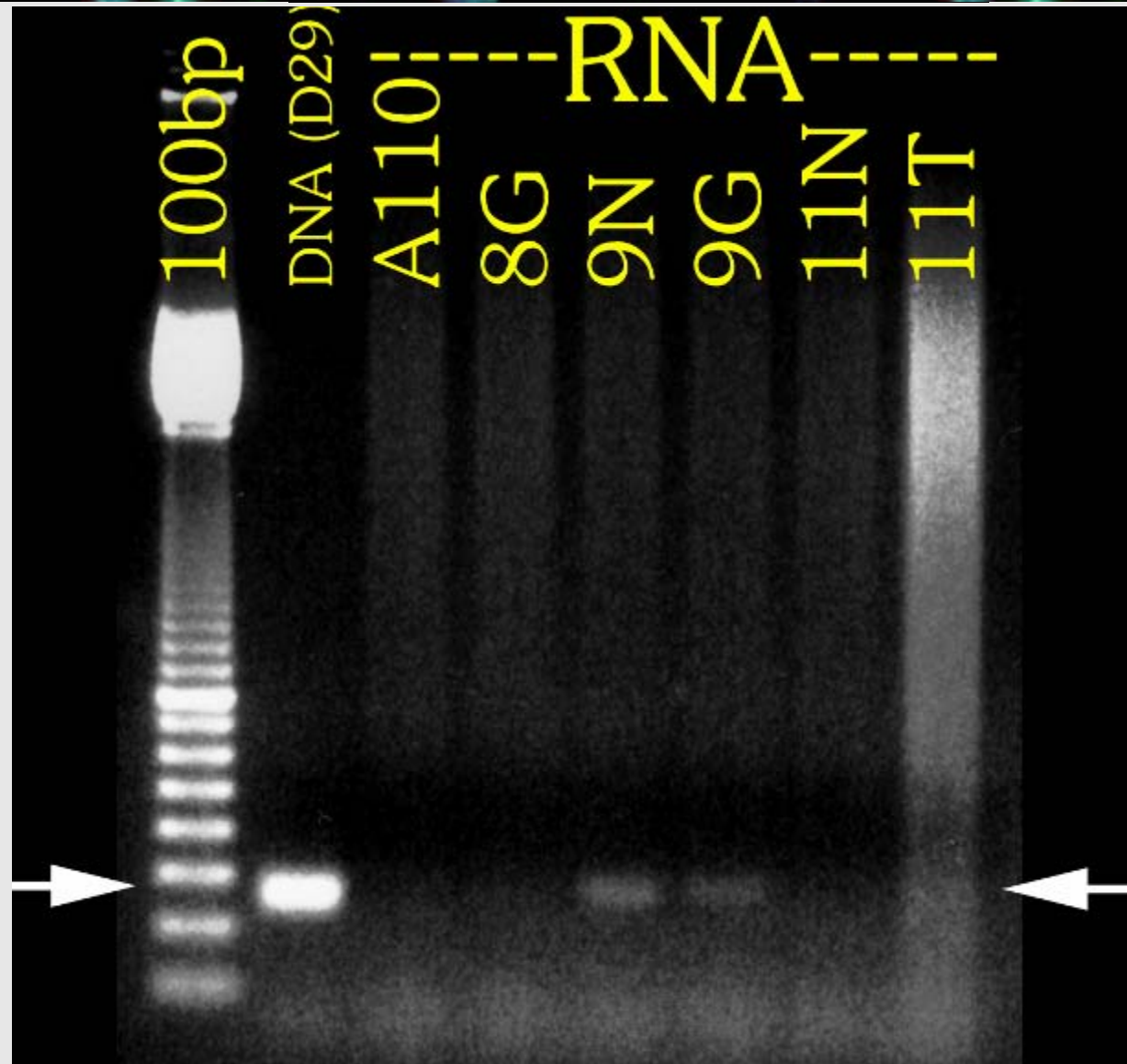
Callus | Trees | Susp'n

kb
7
6
5
4
3
2
1.6
1



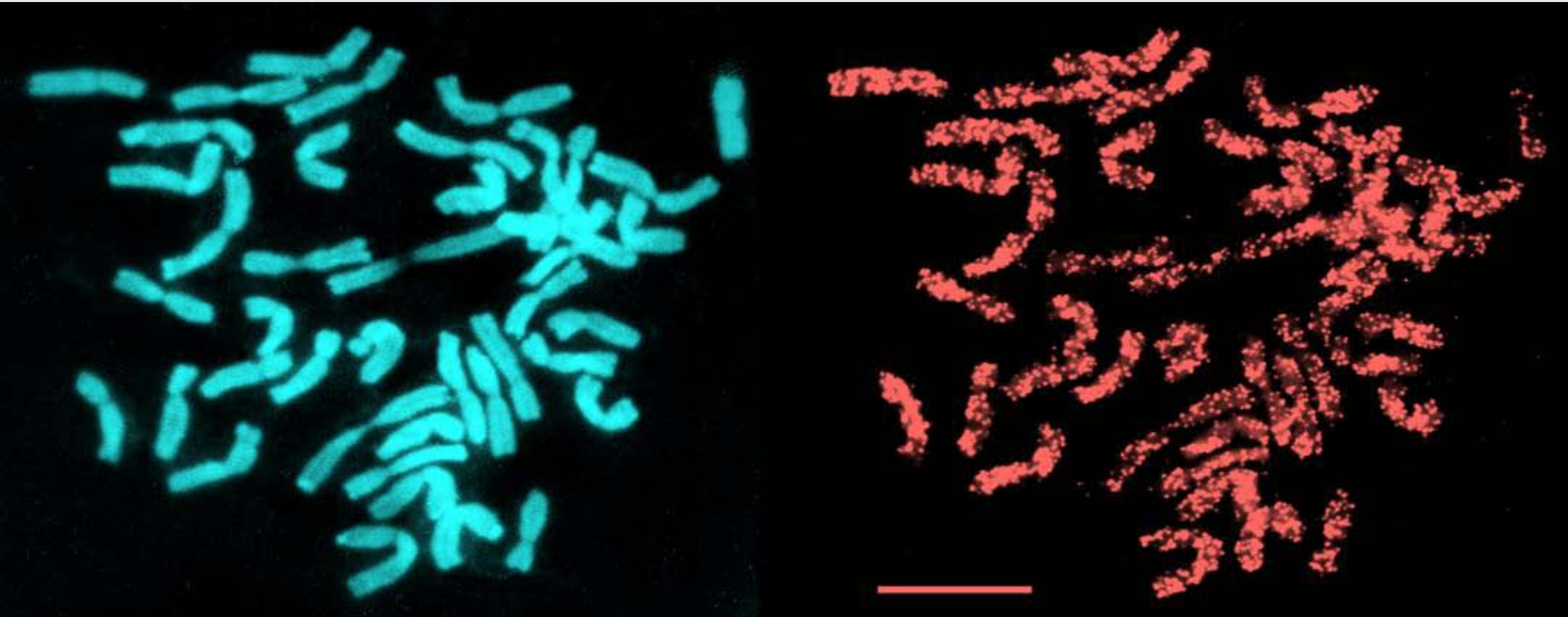
Transcriptional Activity

- ☛ *Copia* mRNA is present in tissue culture
- ☛ Analysis by RT-PCR
- ☛ 260bp product in some lines



Modulation of Methylation

Anti-methylcytosine antibody





Evolution → Epigenetics → Development

Phenotype

Multiple abnormalities

Genetic changes
non-reverting

Changes seen, some reverting

(Male/Female)
Normal Differentiation

Cause

Chromosomal loss, deletion or
translocation

Gene mutation / base pair
changes

Telomere shortening

Retro)transposon insertion

Retrotransposon activation

SSR expansion

Methylation

Heterochromatinization

Chromatin remodelling

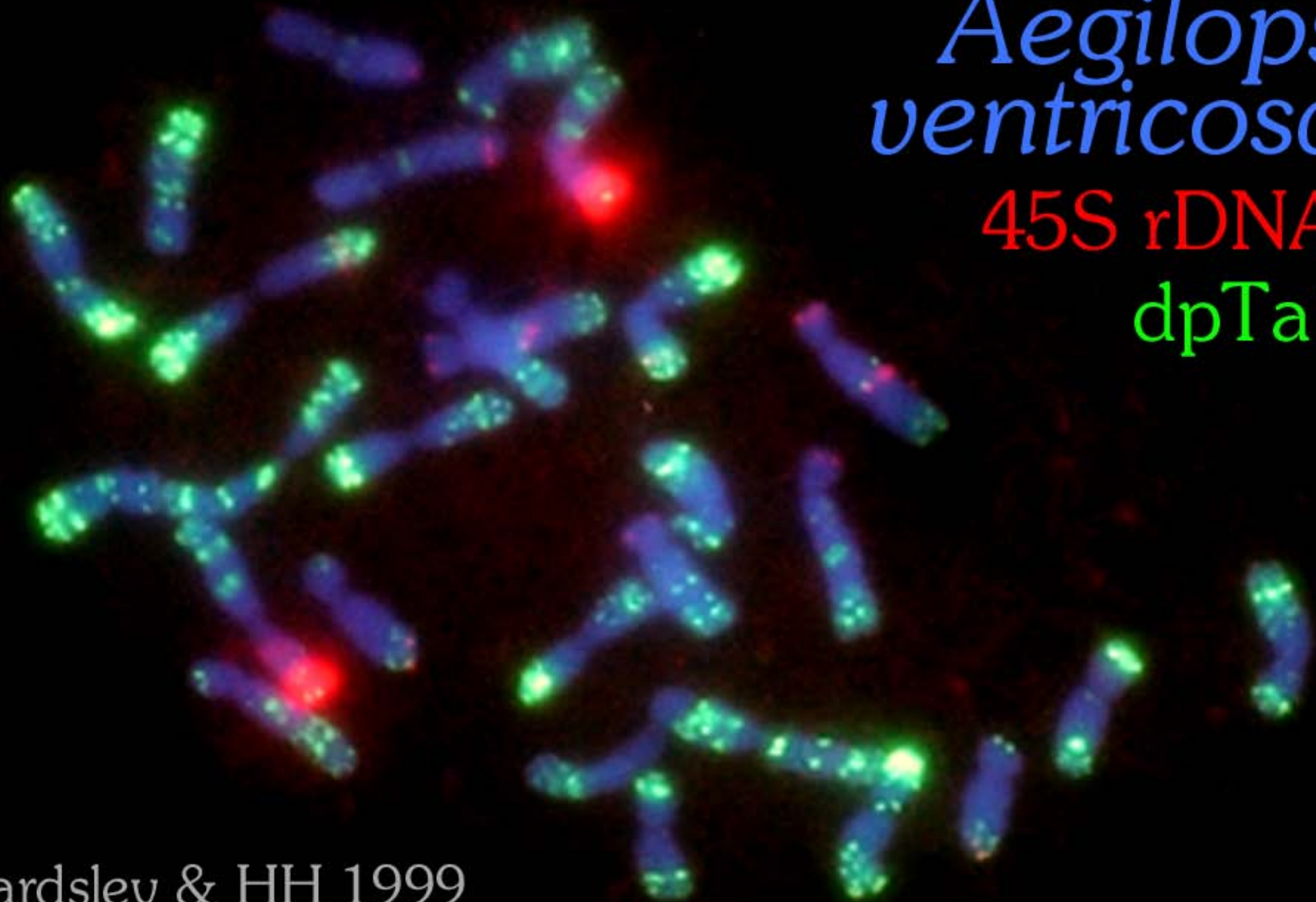
Histone modification



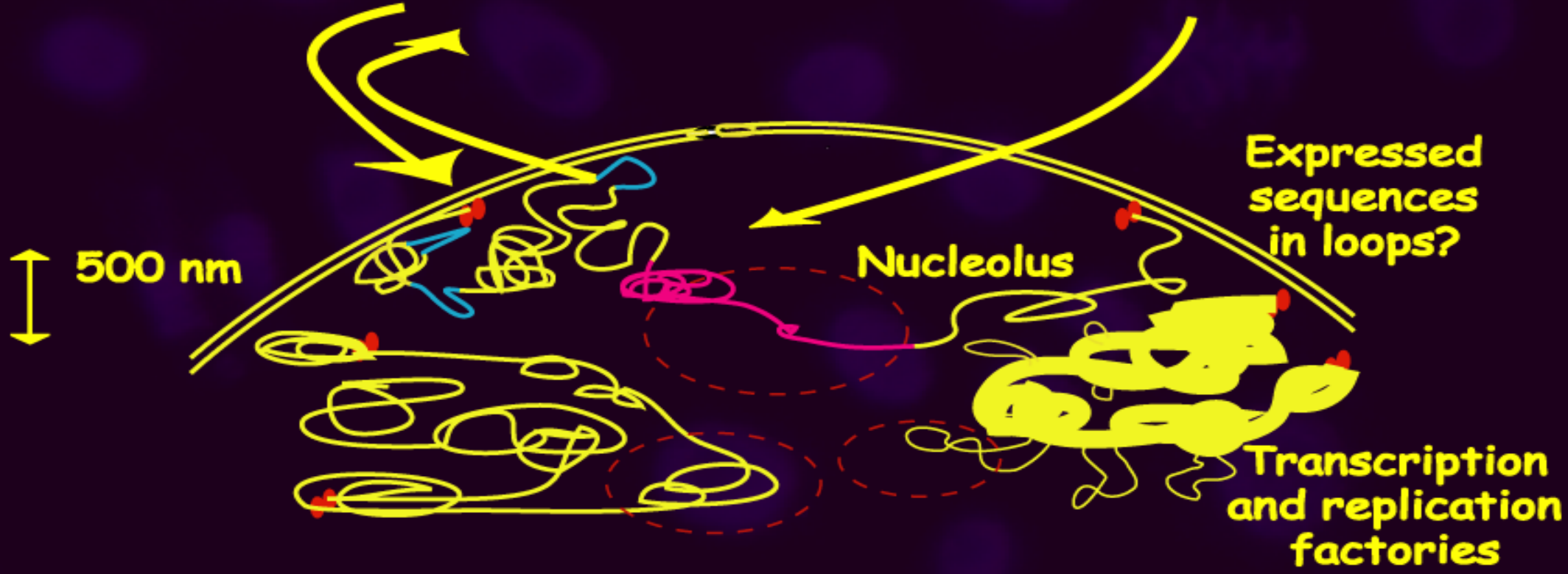
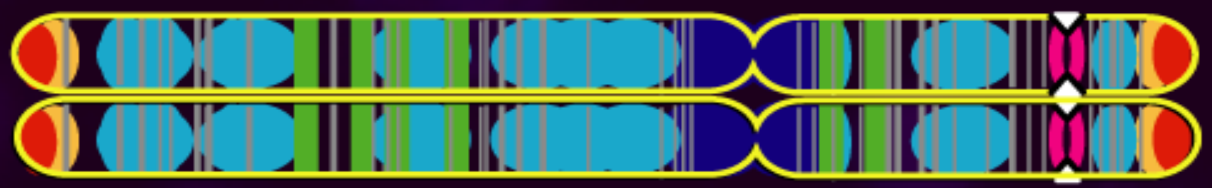
*Aegilops
ventricosa*

45S rDNA

dpTa1



$(TTTAGGG)_n \dots N \times 106s \dots (TTTAGGG)_n$



Super-Domestication: United Nations

Millennium Development Goals

- ✔ Goal 1 – Eradicate extreme poverty and hunger
- ✔ Goal 2 – Achieve universal primary education
- ✔ Goal 3 – Promote gender equity and empower women
- ✔ Goal 4 – Reduce child mortality
- ✔ Goal 5 – Improve maternal health
- ✔ Goal 6 – Combat HIV/AIDS, malaria and other diseases
- ✔ Goal 7 – Ensure environmental sustainability
- ✔ Goal 8 – Develop a global partnership for development

Convention on Biodiversity (“Rio Convention”): inventory the world's diversity

... needs for agriculture: not the only reasons for genomics

Moral imperative not to destroy that we have in the world



Thank You For Shopping



banana genotype was rather flavourless and starchy (Prata Ana), but two others emphasized what we in the temperate countries miss: Garantida II with citrus flavours overlaying a sweet smooth texture, while Caipira had a more savoury and vanilla custard taste. But the



Banana genotypes



ESPRESSO
TALL/GRANDE/VENTI

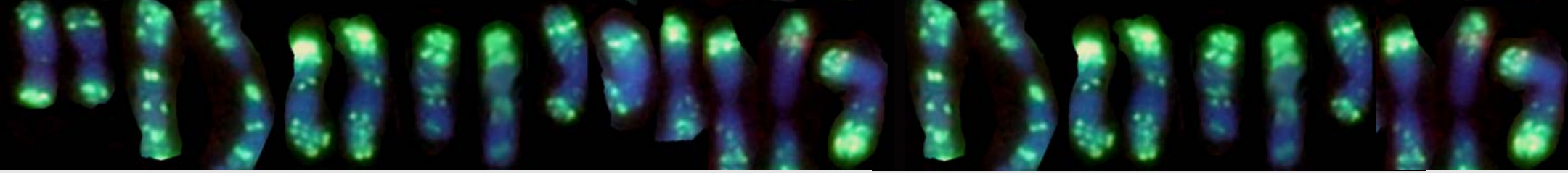
- Espresso
- Caffè Americano
- Caffè Latte
- Cappuccino
- Caramel Macchiato
- Vanilla Latte
- Skinny Vanilla Latte
- Caffè Mocha
- White Chocolate Mocha
- Espresso Truffle
- Pike
- Today
- Caffè

Add Extra Shot/Syrup

TEA - Brewed Hot or Shaken Ice
TALL/GRANDE/VENTI

menu: Blog/newsources.com





Conventional Breeding

- ☞ Cross the best with the best and hope for something better

Superdomestication

- ☞ Decide what is wanted and then plan how to get it
- ☞ - variety crosses
- ☞ - mutations
- ☞ - genepool
- ☞ - genes

FINANCIAL TIMES

From Prof Donald Braben and others.

Sir, We the undersigned scientists write to draw attention to a neglected aspect of the current economic crisis. Robert Solow won the Nobel Prize in economics in 1987 for his 1950s discovery that *technical change* was the biggest source of growth, a discovery that seems to have been forgotten.

Scientific advances are not predictable.

University, Nobel laureate
Pat Heslop-Harrison, University of
Leicester
Steve Howdle, University of

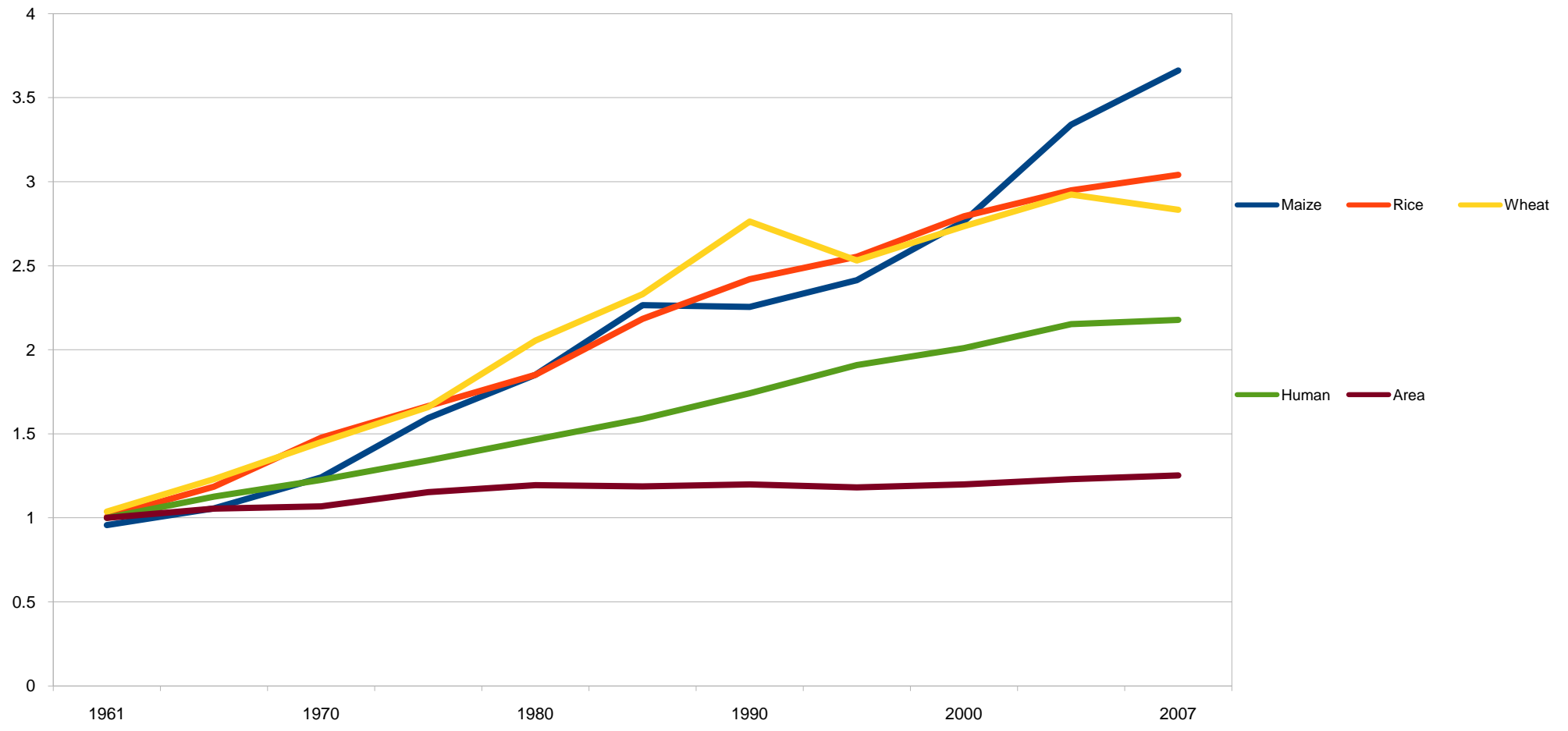
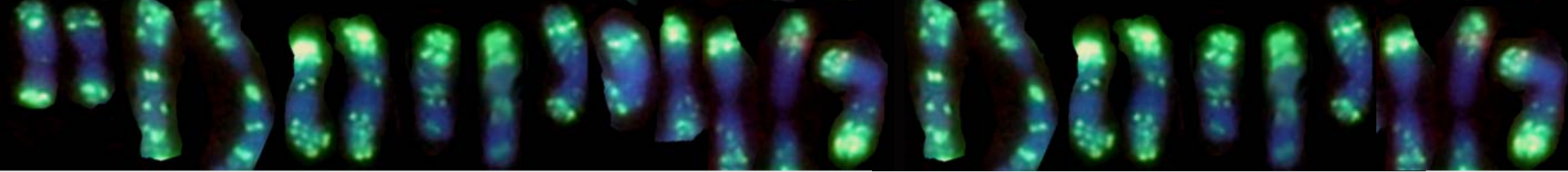
Future – Pollution and land use





Threats to sustainability: no different for 10,000 years

- ☞ Habitat destruction
- ☞ Climate change (abiotic stresses)
- ☞ Diseases (biotic stresses)
- ☞ Changes in what people want
- ☞ Blindness to what is happening
- ☞ Unwillingness to change



year (millions)

item	2007
People	6,602
Maize	785
Rice, paddy	652
Wheat	607
Potatoes	322
Sugar beet	248
Cassava	228
Soybeans	216
Oil palm fruit	192
Barley	136
Sweet potatoes	126
Tomatoes	126
Watermelons	93
Bananas	81
Seed cotton	73
Cabbages and other brassicas	69
Grapes	66
Sorghum	65
Onions, dry	64
Apples	64
Oranges	64
Coconuts	55
Yams	52
Rapeseed	49
Cucumbers and gherkins	45
Groundnuts, with shell	35
Plantains	34
Mangoes, mangosteens	33
Eggplants (aubergines)	32
Millet	32

FAO Statistics 2007

All plant crops with >30M tons annual production

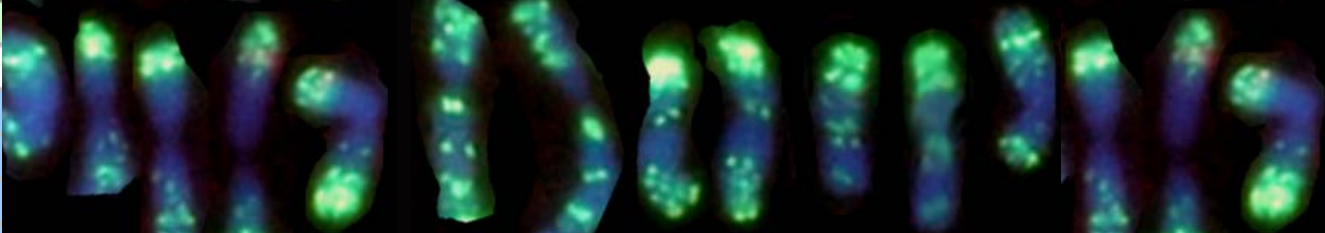
excluding sugar cane and 'other vegetables'

People: WHO

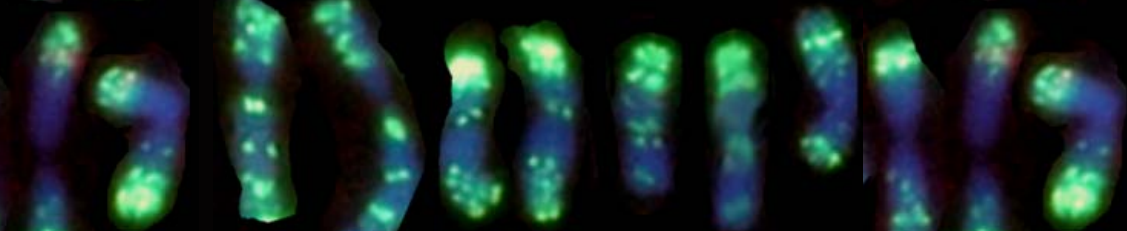
Calories are pretty important – 'let them eat micronutrients' is

year (millions)

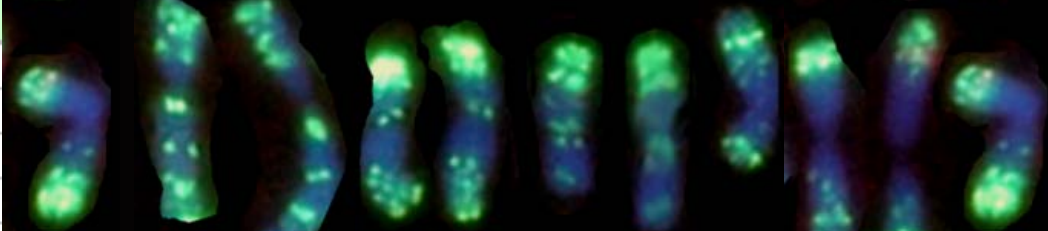
item	1961	2007
People	3,090	6,602
Maize	205	785
Rice, paddy	216	652
Wheat	222	607
Potatoes	271	322
Sugar beet	161	248
Cassava	71	228
Soybeans	27	216
Oil palm fruit	14	192
Barley	72	136
Sweet potatoes	98	126
Tomatoes	28	126
Watermelons	18	93
Bananas	21	81
Seed cotton	27	73
Cabbages and other brassicas	23	69
Grapes	43	66
Sorghum	41	65
Onions, dry	14	64
Apples	17	64
Oranges	16	64
Coconuts	24	55
Yams	8	52
Rapeseed	4	49
Cucumbers and gherkins	10	45
Groundnuts, with shell	14	35
Plantains	13	34
Mangoes, mangosteens, guavas	11	33
Eggplants (aubergines)	7	32
Millet	26	32



	year (millions)		
item	1961	2007	2007/1961
People	3,090	6,602	2.1
Maize	205	785	3.8
Rice, paddy	216	652	3.0
Wheat	222	607	2.7
Potatoes	271	322	1.2
Sugar beet	161	248	1.5
Cassava	71	228	3.2
Soybeans	27	216	8.0
Oil palm fruit	14	192	13.7
Barley	72	136	1.9
Sweet potatoes	98	126	1.3
Tomatoes	28	126	4.5
Watermelons	18	93	5.2
Bananas	21	81	3.9
Seed cotton	27	73	2.7
Cabbages and other brassicas	23	69	3.0
Grapes	43	66	1.5
Sorghum	41	65	1.6
Onions, dry	14	64	4.6
Apples	17	64	3.8
Oranges	16	64	4.0
Coconuts	24	55	2.3
Yams	8	52	6.5
Rapeseed	4	49	12.3
Cucumbers and gherkins	10	45	4.5
Groundnuts, with shell	14	35	2.5
Plantains	13	34	2.6
Mangoes, mangosteens, guavas	11	33	3.0
Eggplants (aubergines)	7	32	4.6
Millet	26	32	1.2



item	year (millions)		
	1961	2007	2007/1961
Oil palm fruit	14	192	13.7
Rapeseed	4	49	12.3
Soybeans	27	216	8.0
Yams	8	52	6.5
Watermelons	18	93	5.2
Onions, dry	14	64	4.6
Eggplants (aubergines)	7	32	4.6
Tomatoes	28	126	4.5
Cucumbers and gherkin	10	45	4.5
Oranges	16	64	4.0
Bananas	21	81	3.9
Maize	205	785	3.8
Apples	17	64	3.8
Cassava	71	228	3.2
Rice, paddy	216	652	3.0
Cabbages and other brassicas	23	69	3.0
Mangoes, mangosteens, guavas	11	33	3.0
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People	3,090	6,602	2.1
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Grapes	43	66	1.5
Sweet potatoes	98	126	1.3
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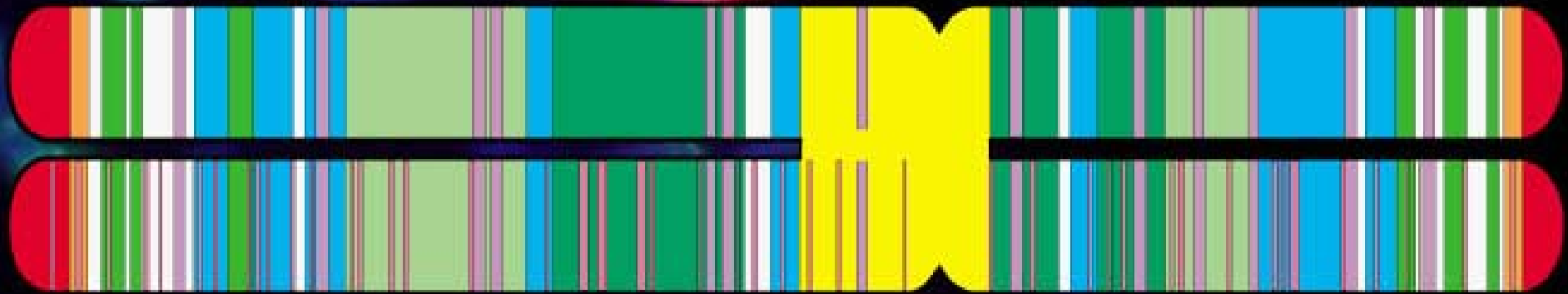


A horizontal strip at the top of the slide shows several pairs of plant chromosomes. The chromosomes are stained with a blue dye, and specific regions are highlighted with a bright green fluorescence, likely representing DNA markers or specific genes of interest.

Plant Genome Evolution and Diversity

1. Genomes and genomics
2. Markers from DNA
3. Diversity and its use
4. Challenges and breeding

The Linear Chromosome

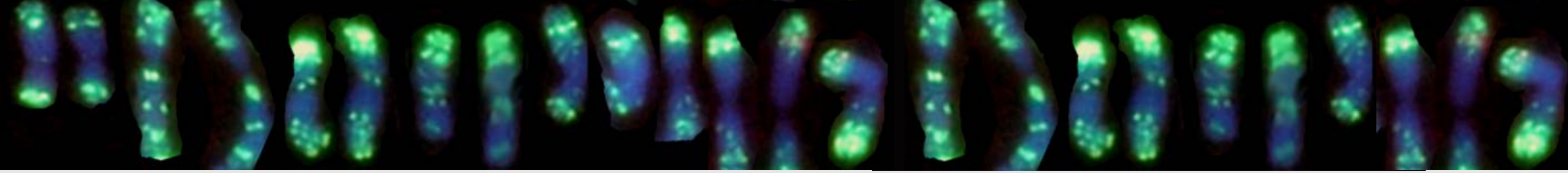


 Tandem repeats

 Retroelements
Simple sequence repeats

 Terminal repeats

 Genes



CytoGenomics ...

- ☛ The genepool has the diversity to address these challenges ...
- ☛ New methods to exploit and characterize germplasm let use make better and sustainable use of the genepool

Species evolution

Polyploidy **Diversity, Genes and Genomics:**

Sequence evolution

Chromosome evolution

Genome diversity

Centromeres/mitosis

Nuclear architecture

Epigenetics

Chromatin

Meiosis/recombination

From Models to Application

Origins

Phylogeny

Diversity

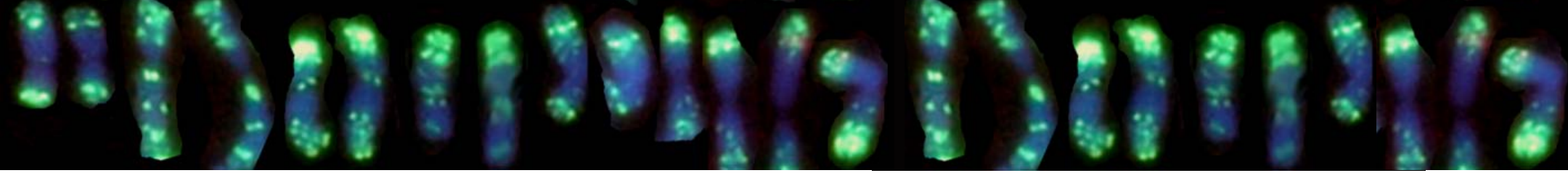
Introgression

Selection

Pre-Breeding

Propagation

Varieties



Plant Molecular Cytogenetics

Pat Heslop-Harrison

phh4@le.ac.uk

www.molcyt.com User&PW = 'visitor'

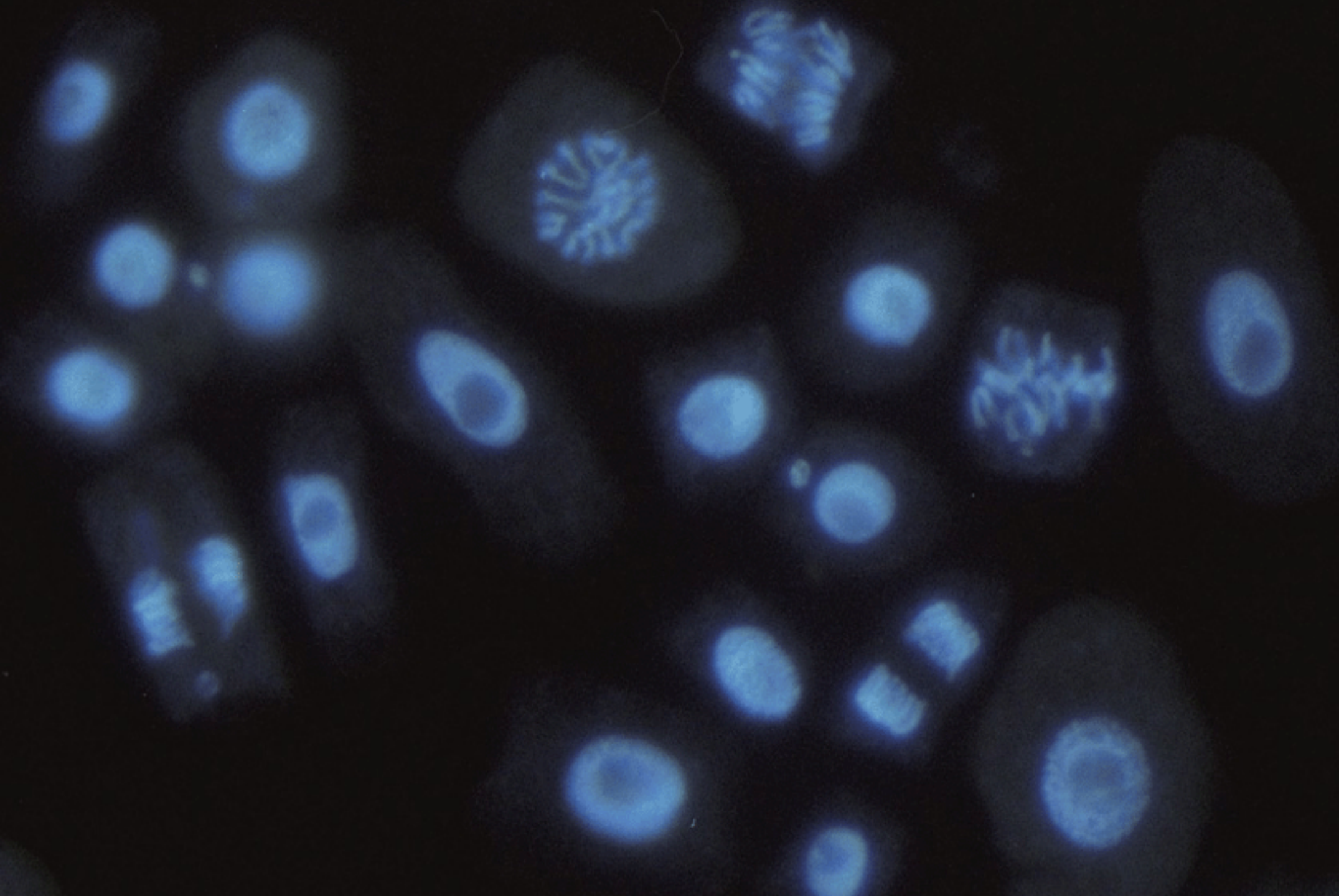
This talk will be linked from homepage

Also: www.sblab.org www.crocusbank.org

www.biobanana.com www.musagenomics.org



- 
- ☛ Weed control
 - ☛ Monocultures
 - ☛ Suitable inputs (nitrogen)
 - ☛ Diseases - co-evolution
 - ☛ Post-harvest losses
 - ☛ Alien species



Do we need change?

Do we need faster change?

Crop varieties

- High yield
- High quality and safe
- Easy to grow agronomically
- Disease resistant
- Insect/nematode resistant
- Efficient water use
- Secure, stable production
- Environmentally friendly
 - Not invasive

Triticale:
a wheat x rye
hybrid

