

Chromosome Analysis and Molecular Cytogenetics

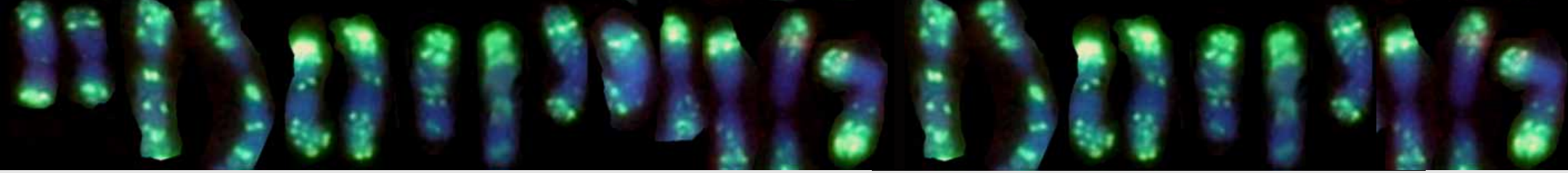
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www.molcyt.com

www.molecularcytogenetics.com

UserID/PW: 'visitor'



Molecular Cytogenetics

☞ Chromosomes

- Genome sizes
- Structure
- Components

☞ Applications

- Evolution
- Hybrids
- Aneuploids and substitutions



O₁

E₁ V₄

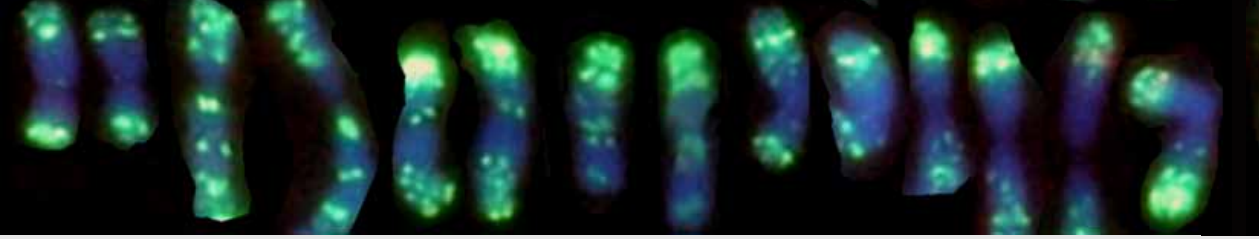
L₁ U₁ T₁

O₁ Z₁

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.

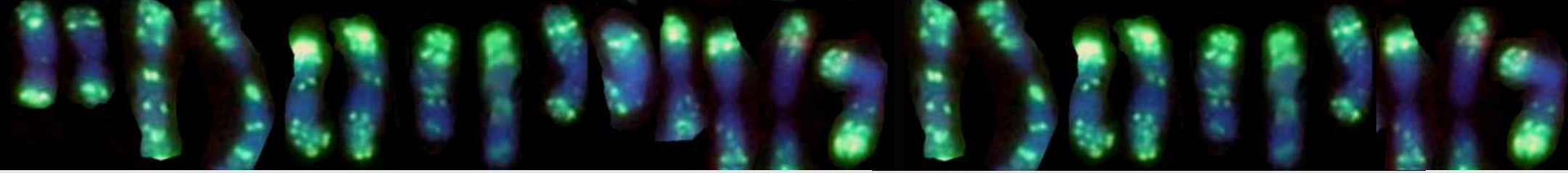




Nothing in Biology
Makes Sense
Except in the
Light of Evolution

Theodosius Dobzhansky (1900-1975)

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



Molecular Cytogenetics

☞ Lecture

- 10.05-11.00
- 11.10-12.00

☞ Microscopy (Adrian 264 near computer room)



OBJECTIVES

Fundamental and Practical

- ☞ Explain major structures and features of the DNA in plant 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

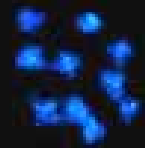


OBJECTIVES

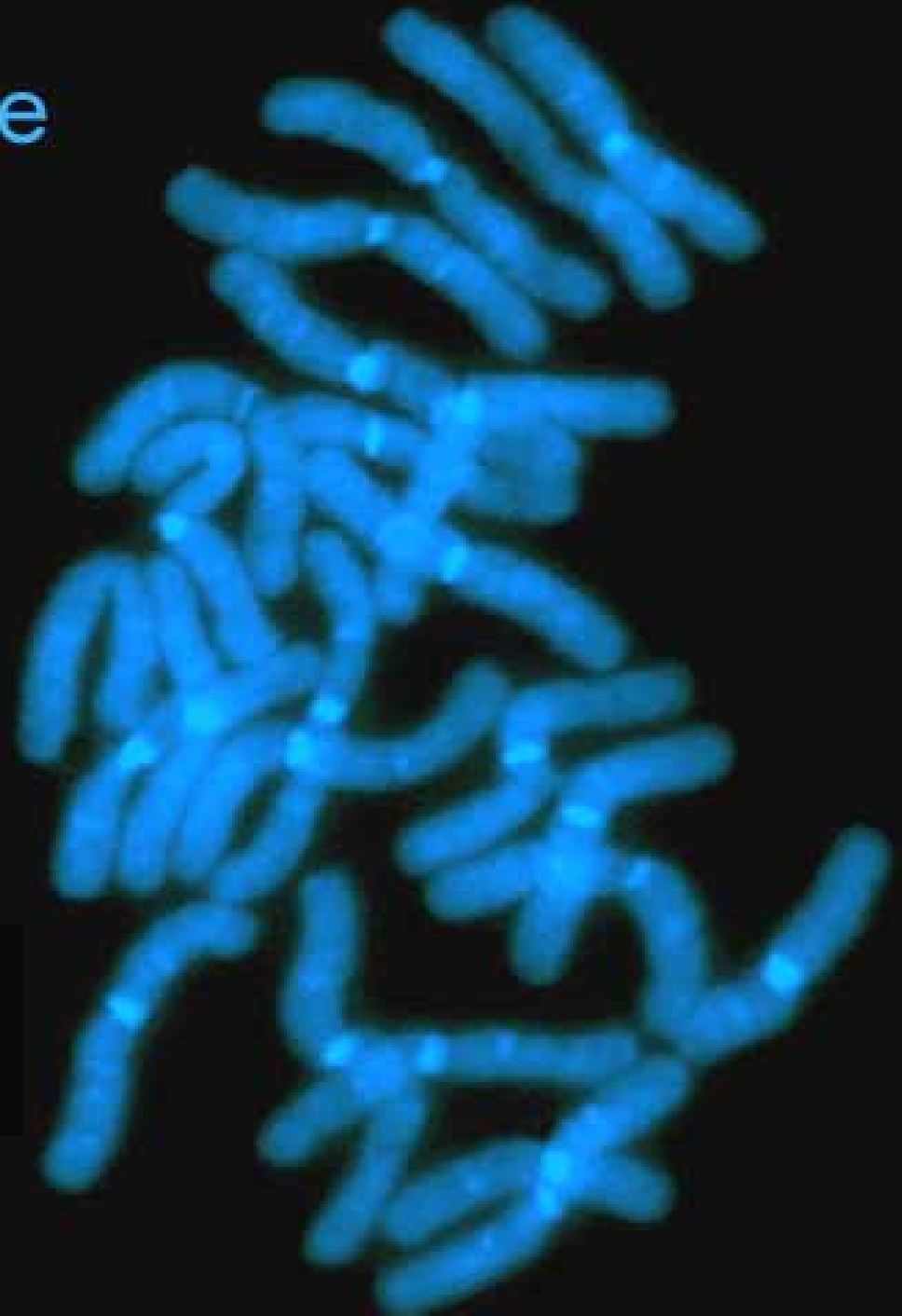
Fundamental and Practical

- 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

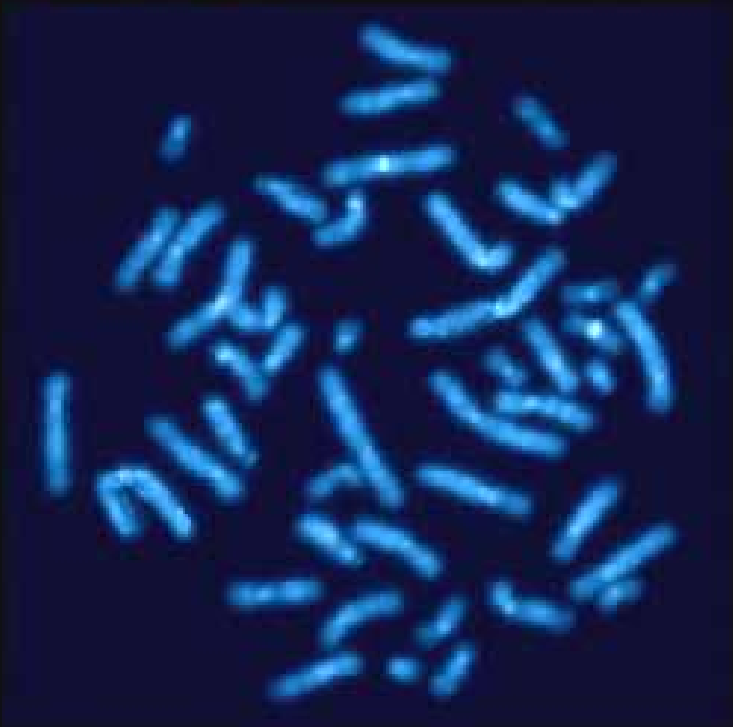
Arabidopsis

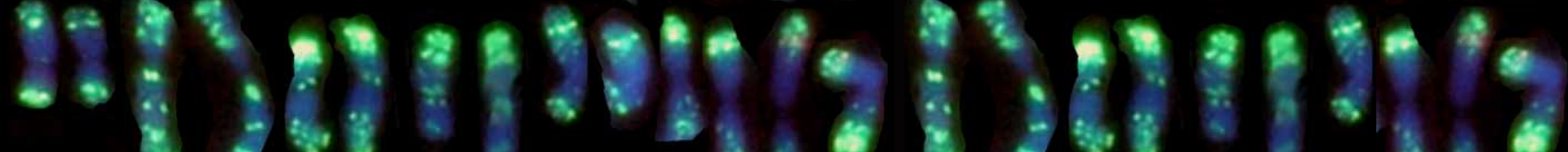


Pine



Human

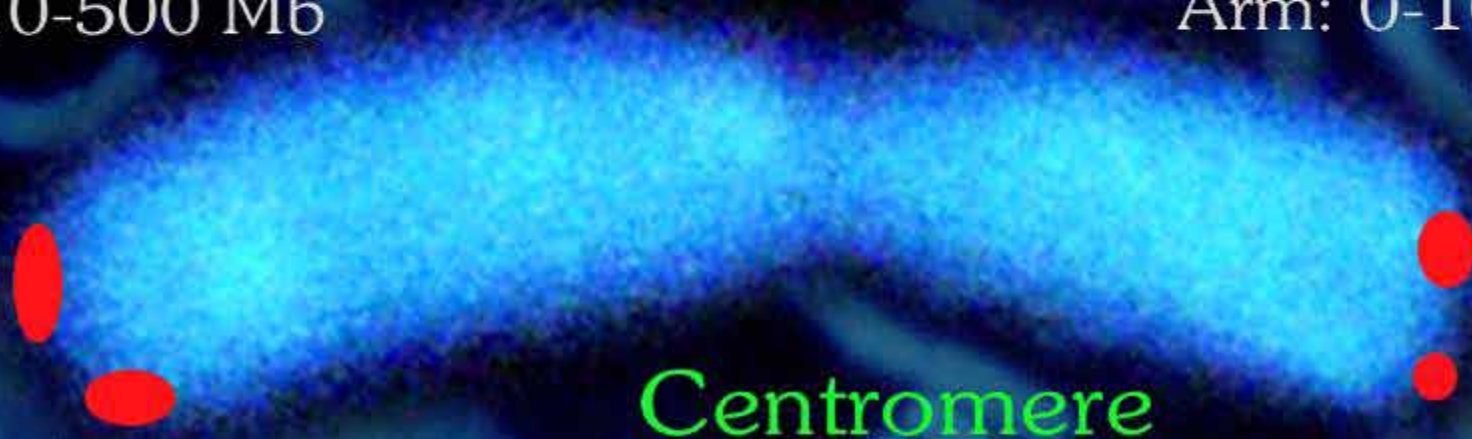




Components of a Chromosome

Arm: 10-500 Mb

Arm: 0-1000 Mb



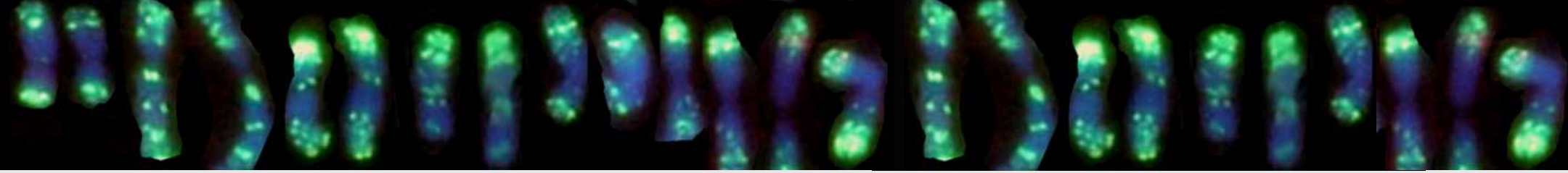
Telomeres

Centromere

Genes!

Packaging
- Division
- Organization

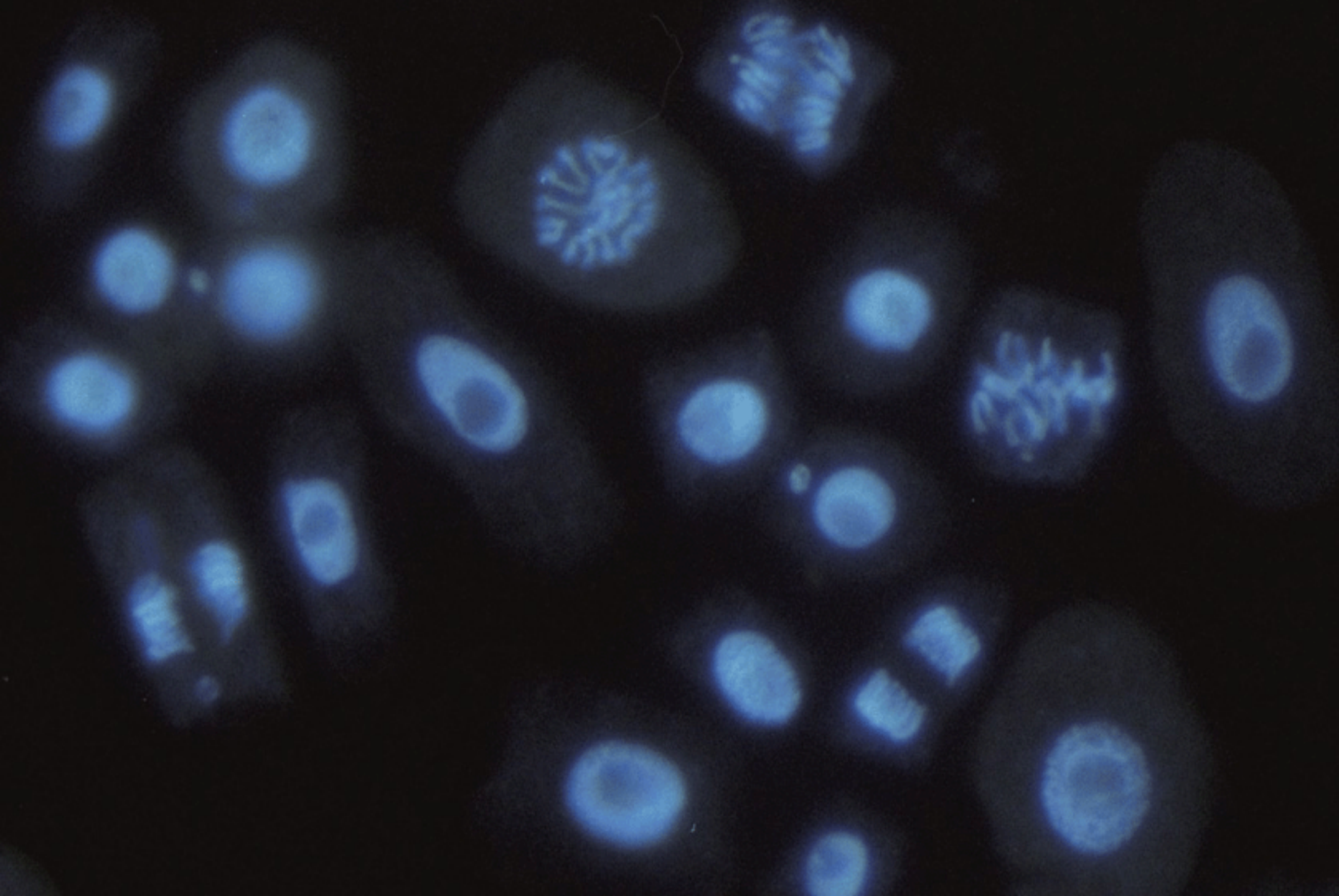
Replication and Transcription
- Enzyme access
- Regulation



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 *χρῶμα* (*chroma*, colour) and *σῶμα* (*soma*, body) due to its capacity to be stained very strongly



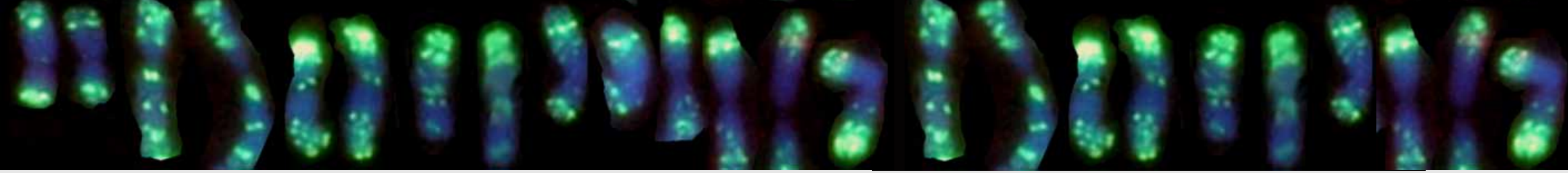


Chromosome function

- ☞ Replicate
- ☞ Divide
- ☞ Condense
- ☞ Express genes
- ☞ Regulate above functions
- ☞ Recombine

- ☞ Mutate
- ☞ Evolve

- ☞ Package DNA
- ☞ Maintain stability



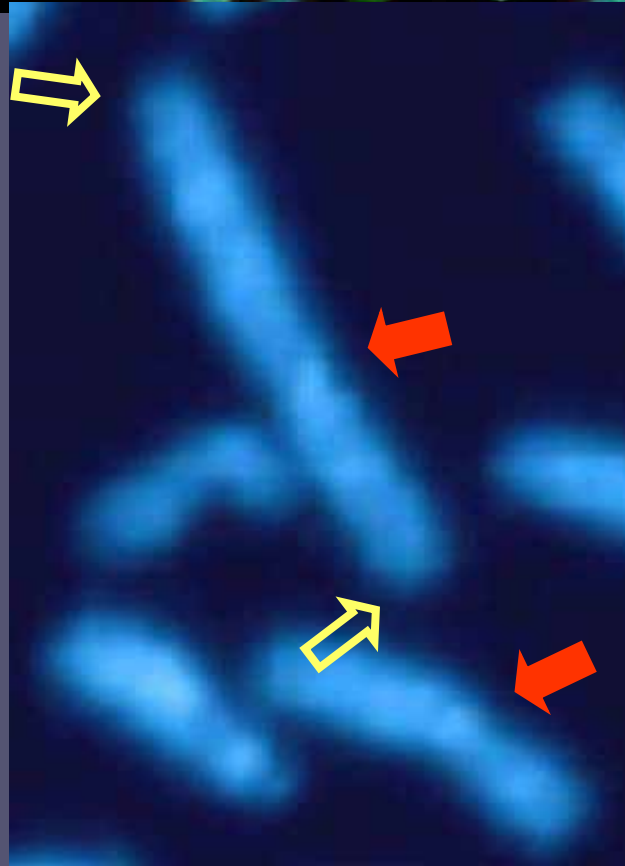
What does the genome do?

- ☛ Contains the genes
- ☛ Partitions the genes among chromosomes
- ☛ Provides a stable structure for genes, controlling sequences and other DNA

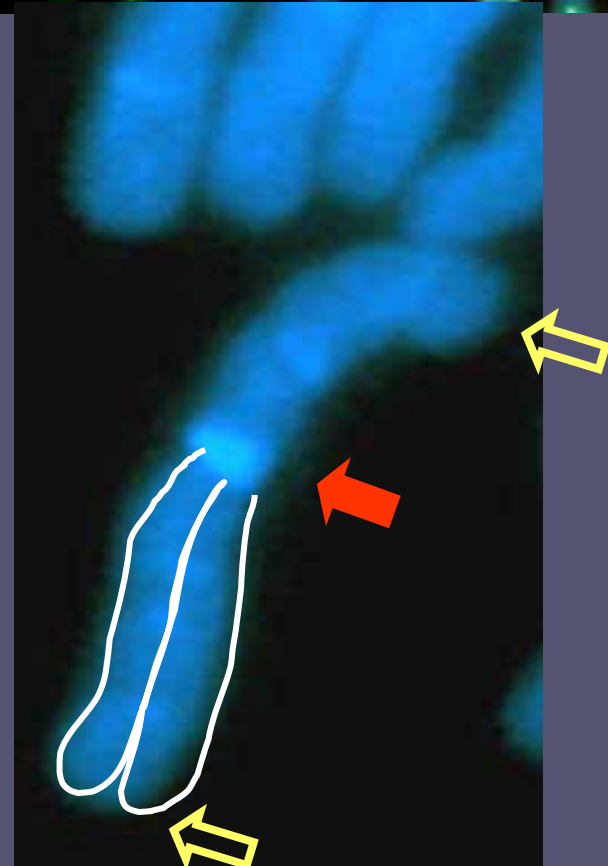
Somatic metaphase chromosomes



Arabidopsis



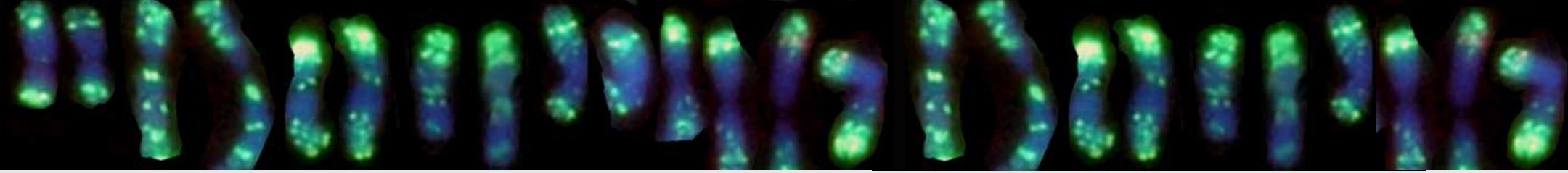
Human



Pine

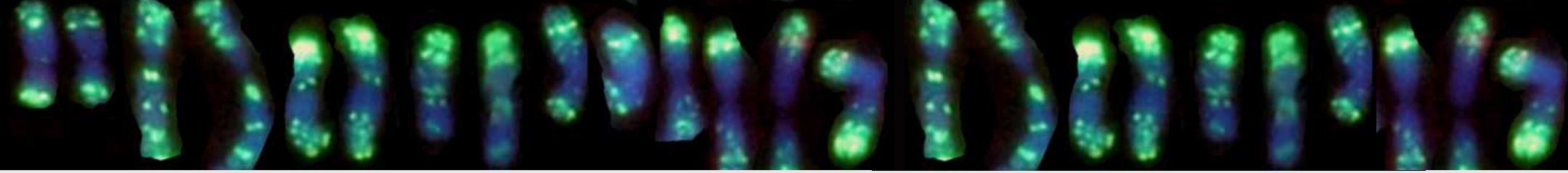
Centromere

Telomere



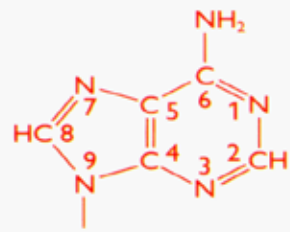
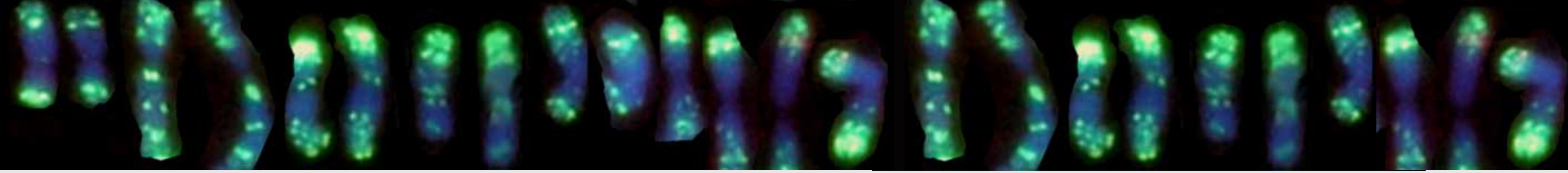
Chromosomes

- They carry the genetic information
- Their behaviour determines the mechanism of inheritance
- Their morphology relates to function
- Their changes relate to evolution
- Their changes relate to disease

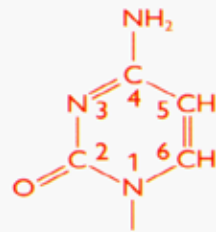


Views of the Chromosome

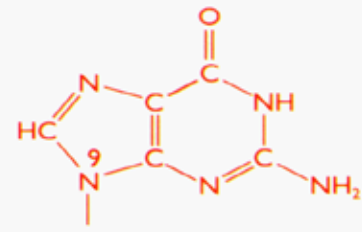
- ☛ The small
- ☛ The chemical
- ☛ The physical/mathematical
- ☛ The evolutionary
- ☛ The big



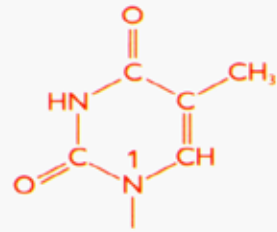
Adenine (A)



Cytosine (C)



Guanine (G)



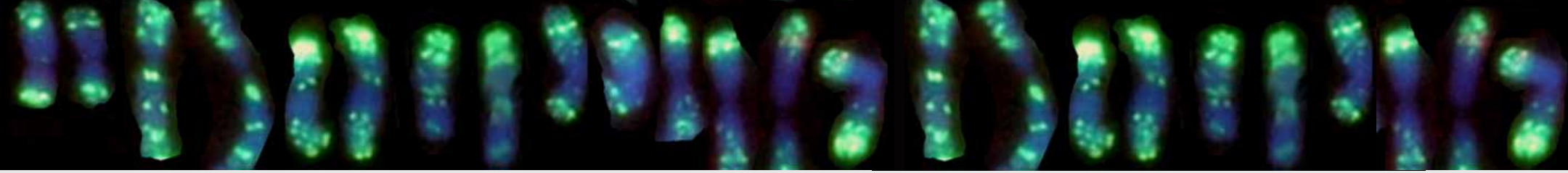
Thymine (T)

Consequences



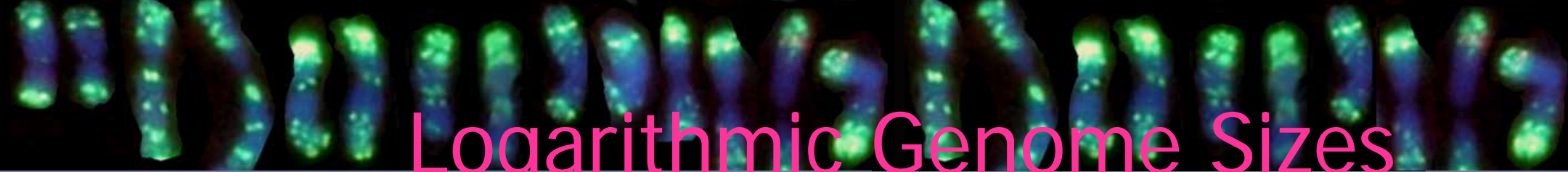
Genome sizes

☛ HIV type 1 Virus	9 750 bp	
☛ Bacteria (<i>E. coli</i>)	4 600 000 bp (4.6 Mbp)	
☛ Yeast (<i>Saccharomyces</i>)	12 Mbp	$2n=c.34$
☛ <i>Genlisea aurea</i>	65 Mbp	$2n=c.52$
☛ <i>Arabidopsis</i>	150 Mbp	$2n=10$
☛ <i>Aesculus</i> (horse chestnut)	200 Mbp	$2n=20$
☛ Rice	389 Mbp	$2n=24$
☛ Banana	550 Mbp	$2n=22 - 33$
☛ Human	3 200 Mbp	$2n=46$
☛ Barley	5 500 Mbp	$2n=14$
☛ Wheat	17 000 Mbp	$2n=42$
☛ <i>Pinus elliotii</i>	24 000 Mbp	$2n=24$
☛ <i>Fritillaria lily</i>	130 000 Mbp	$2n=24$

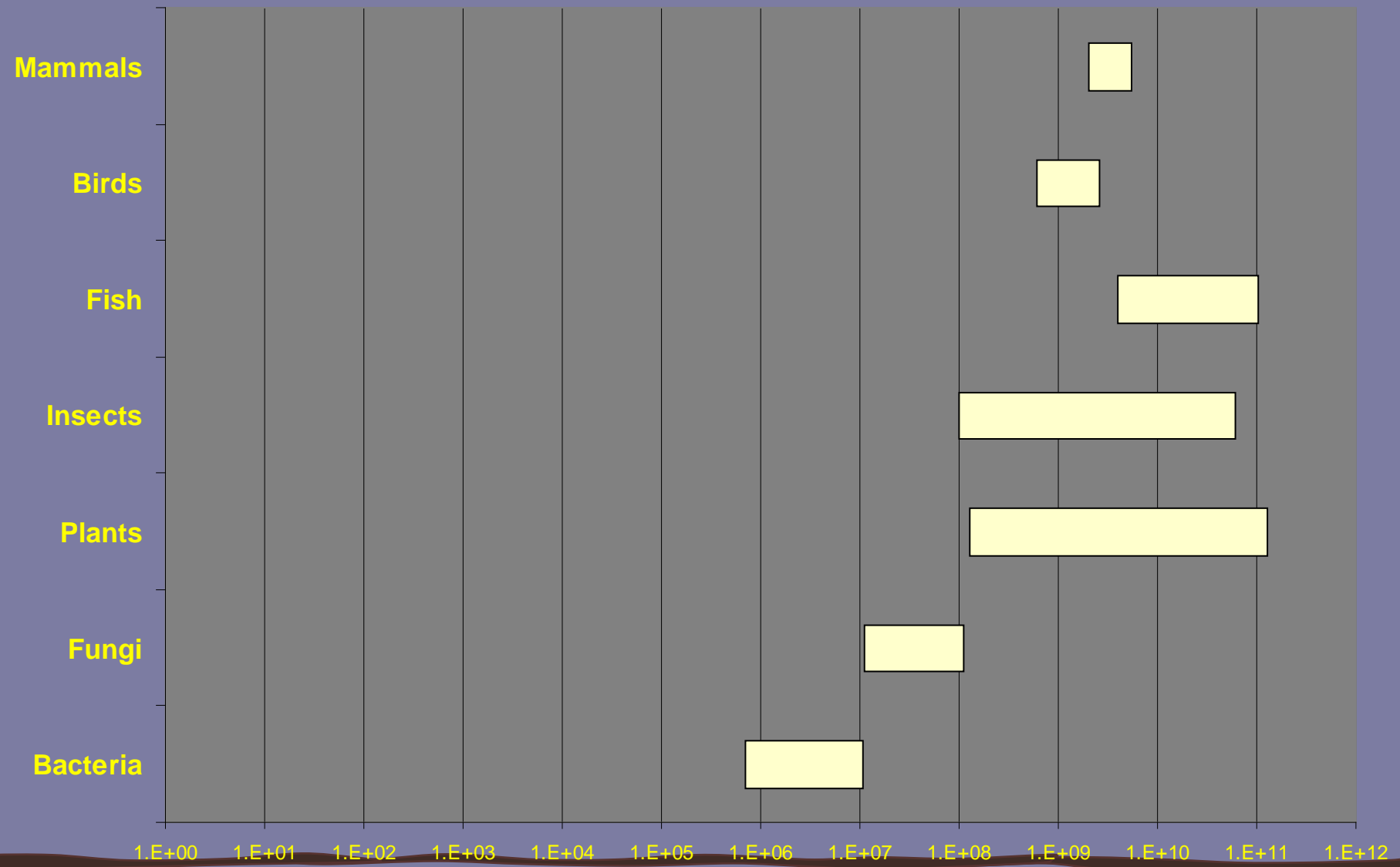


Genome sizes: reading them out base-by-base

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



Logarithmic Genome Sizes

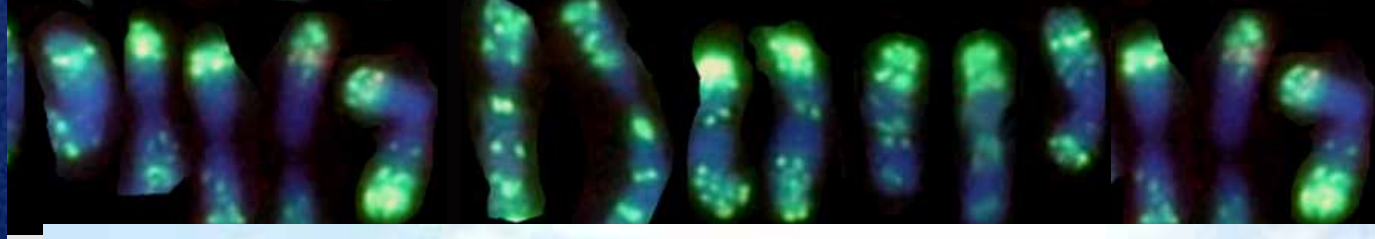
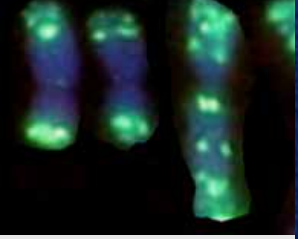


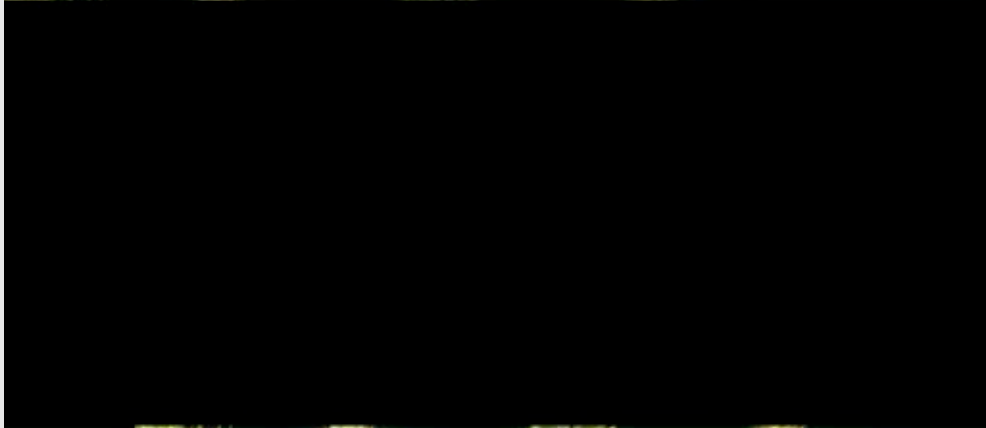
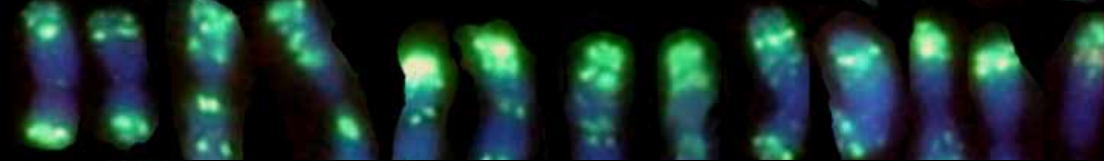
A horizontal strip at the top of the slide shows numerous chromosomes from a karyotype. The chromosomes are stained with a blue dye, and several specific regions are highlighted with a bright green fluorescence, likely representing specific genes or markers used in genomic studies.

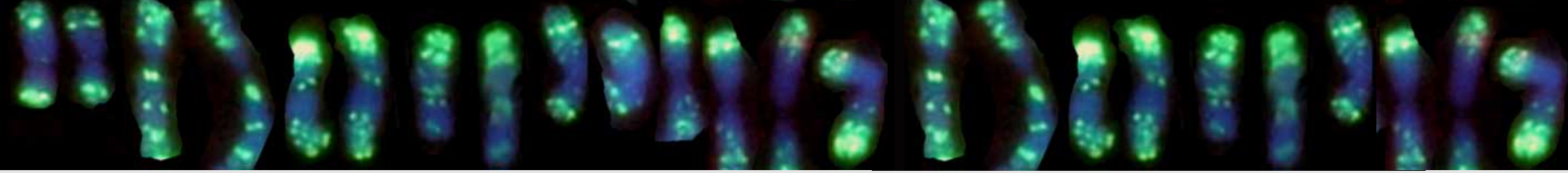
Genome sizes

- ☛ *Arabidopsis* 150 Mbp
- ☛ *Aesculus* (horse chestnut) 200 Mbp

- ☛ Wheat 17 000 Mbp
- ☛ *Pinus elliotii* 24 000 Mbp

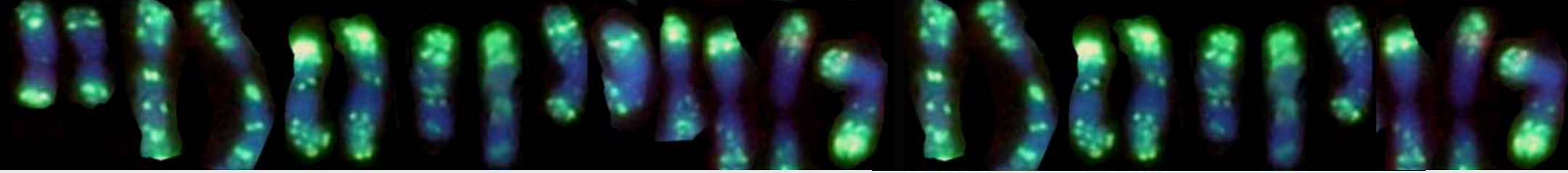






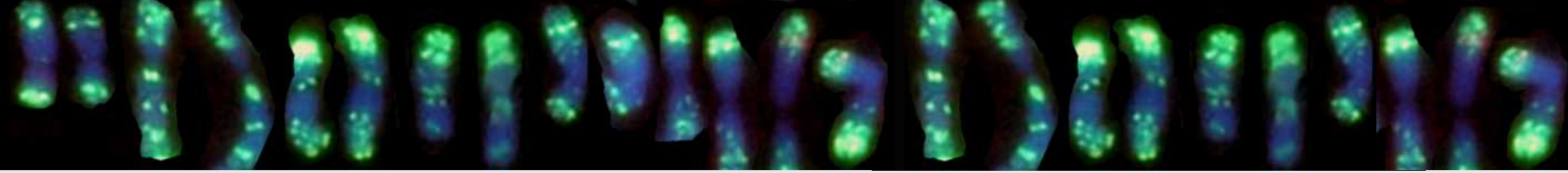
How many genes?

- *Arabidopsis*
- Rice
- Human



How many genes?

☛ <i>Arabidopsis</i>	26,500
☛ Rice	41,000
☛ Human	23,299
☛ <i>Caenorhabditis</i>	20,000



How many genes?

- ☞ In a typical eukaryote:
- ☞ 35,000 genes
- ☞ 300 amino acids
- ☞ 1500 bases inc. introns/part promoter
- ☞ 55,000,000 base pairs or 55 Mbp



Genome sizes

☞ Banana Streak Virus	7 389 base pairs
☞ HIV type 1	9 750 base pairs
☞ <i>Escherichia coli</i>	4.6 Mbp
☞ <i>Saccharomyces</i>	12.1 Mbp
☞ <i>Arabidopsis</i>	140 Mbp
☞ <i>Oryza</i>	400 Mbp
☞ <i>Homo</i>	3 200 Mbp
☞ <i>Hordeum</i>	5 500 Mbp
☞ <i>Triticum aestivum</i>	17 000 Mbp
☞ <i>Fritillaria</i>	130 000 Mbp



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%	3200 Mbp
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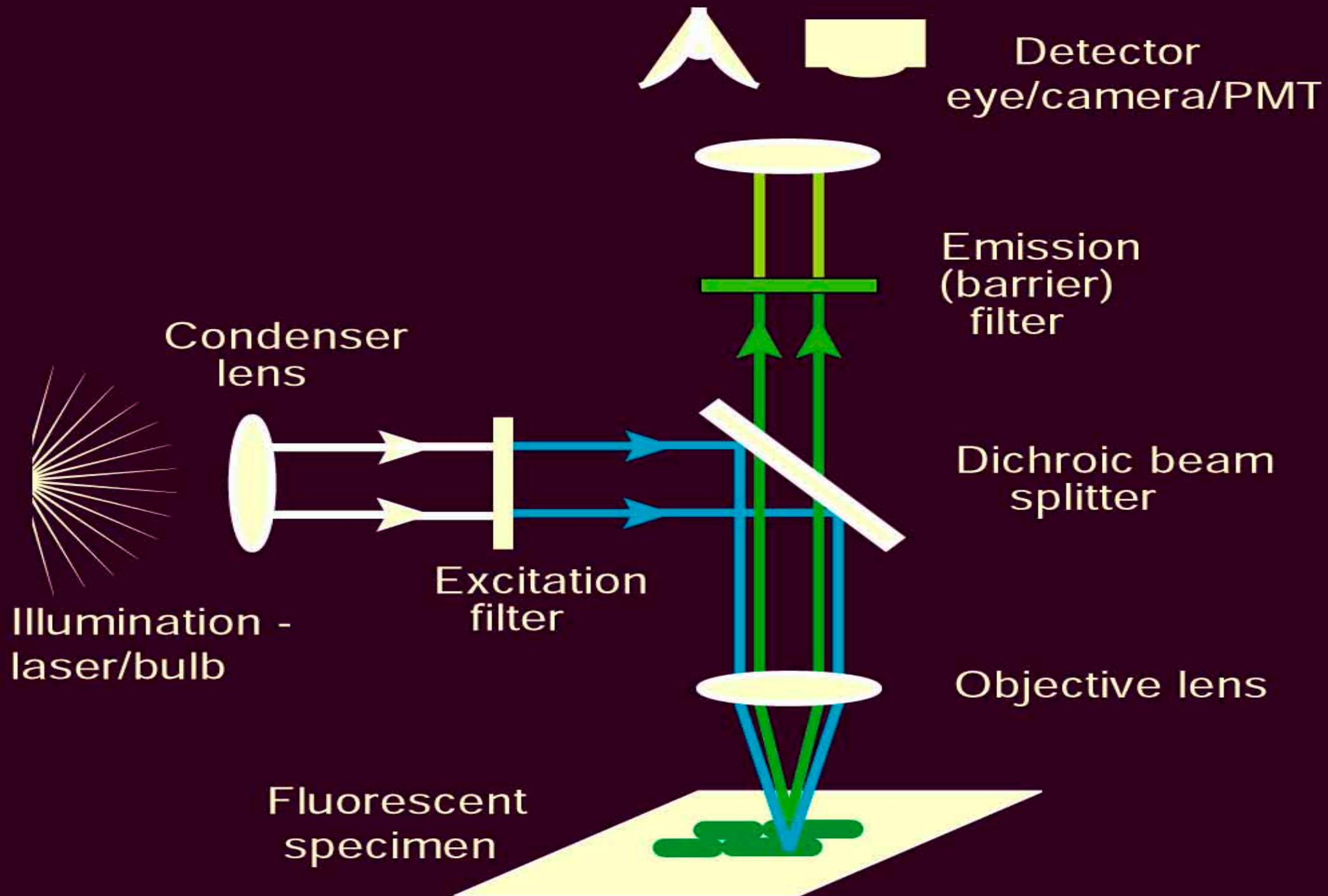


The Genome and Chromosomes

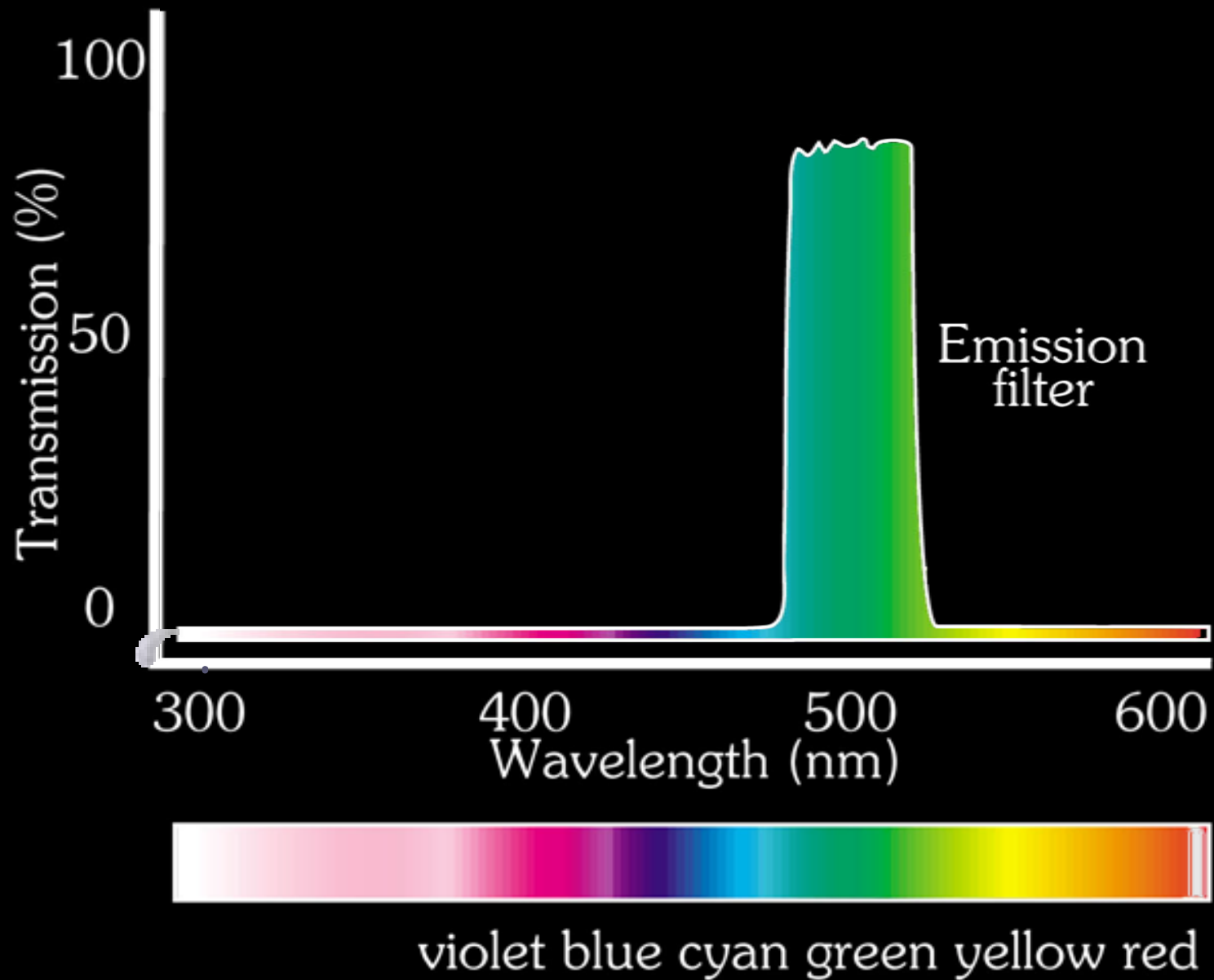
- ☞ The DNA is organized in chromosomes
- ☞ Bacteria have one circular chromosome with the whole genome
- ☞ Eukaryotes have multiple chromosomes
- ☞ Each is a single, linear DNA molecule
- ☞ The DNA is packaged around proteins (histones)
- ☞ The chromosome has special sequences at its ends
- ☞

Fluorescence microscopy

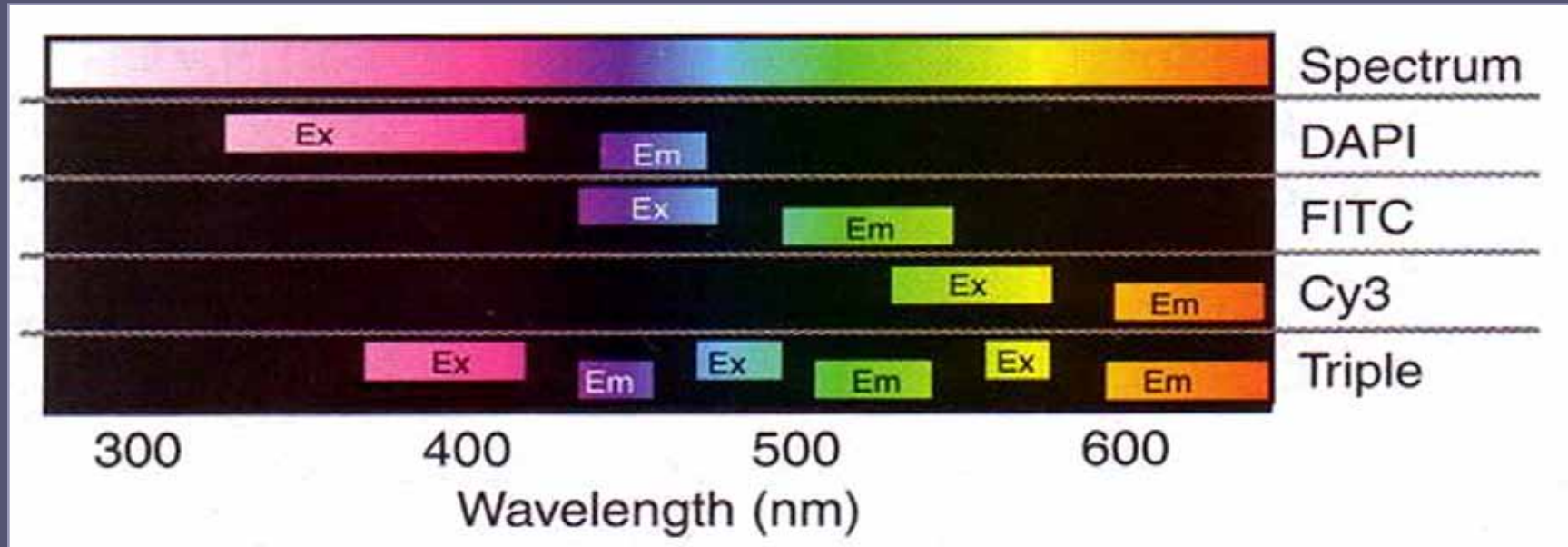




Fluorescence microscope filter properties



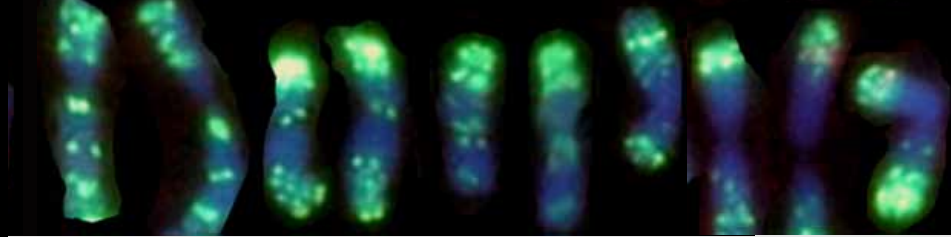
Fluorescence microscopy



Multi-colour FISH:

- Hybridizing two or more DNA probes simultaneously
- Multibandpass filters

Human female
R-bands

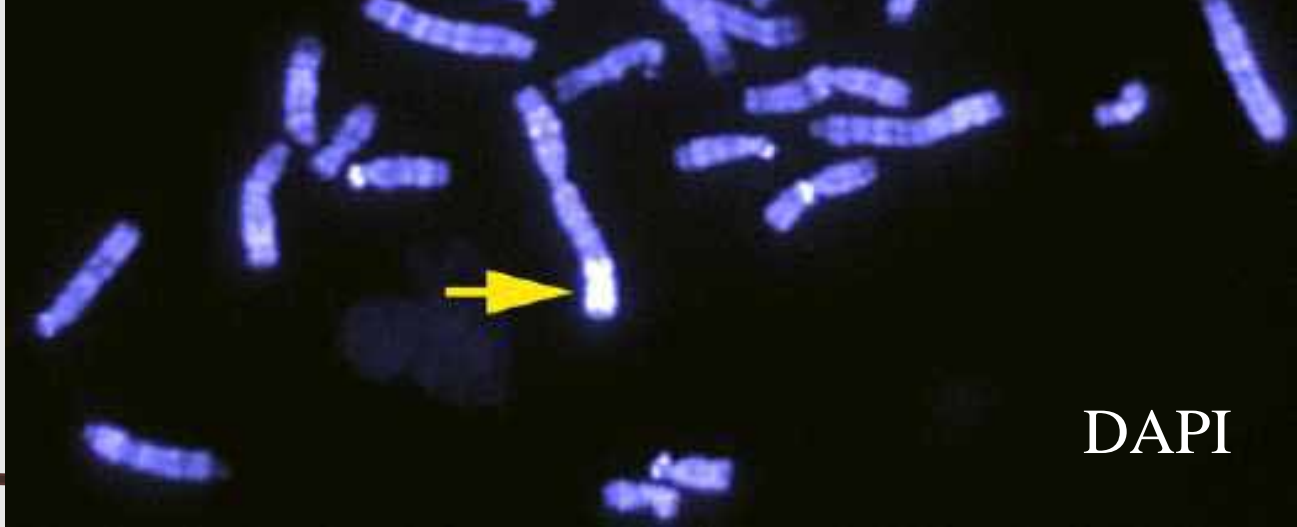
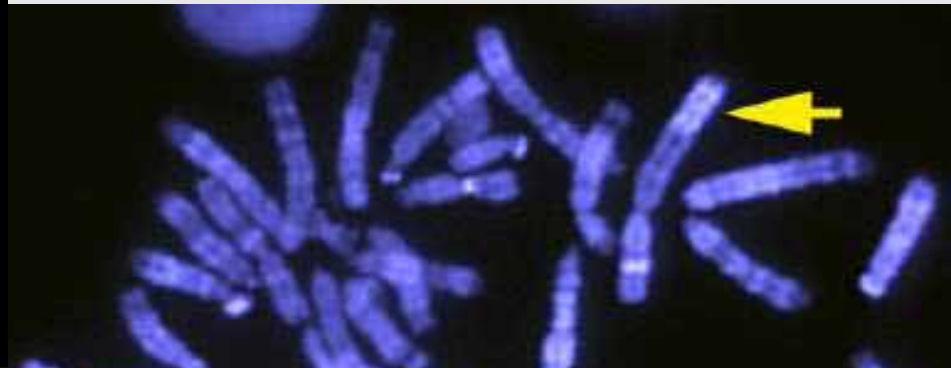


Fluorescent banding

DAPI: AT-rich

ChromomycinA3: GC-rich

Chromomycin A3: binds to GC rich DNA sequences

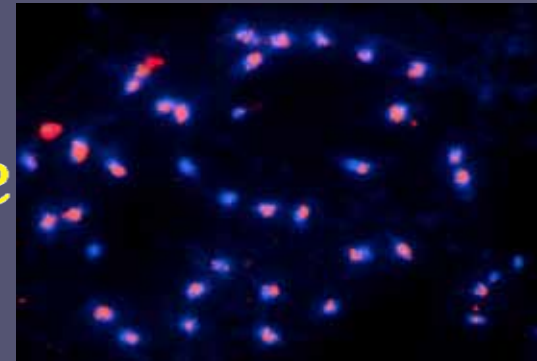
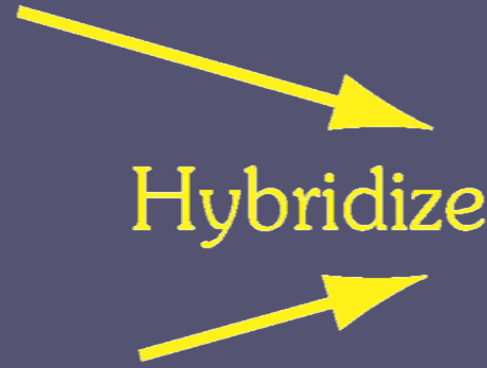
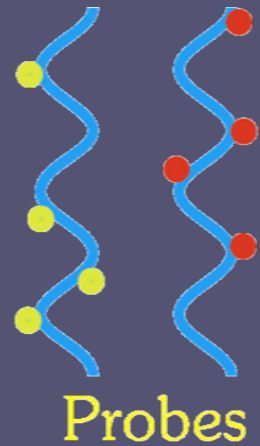


DAPI

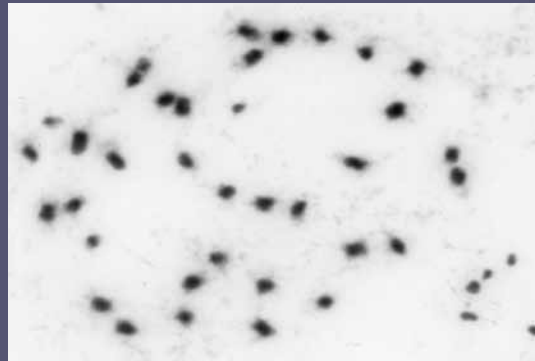
Triticale:
wheat x rye
hybrid



In situ and array hybridization technology



Detection of
Hybridization sites



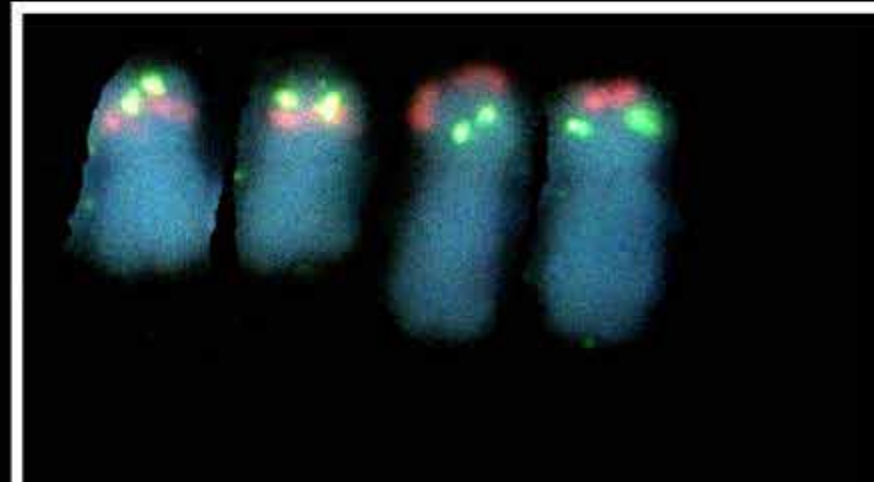
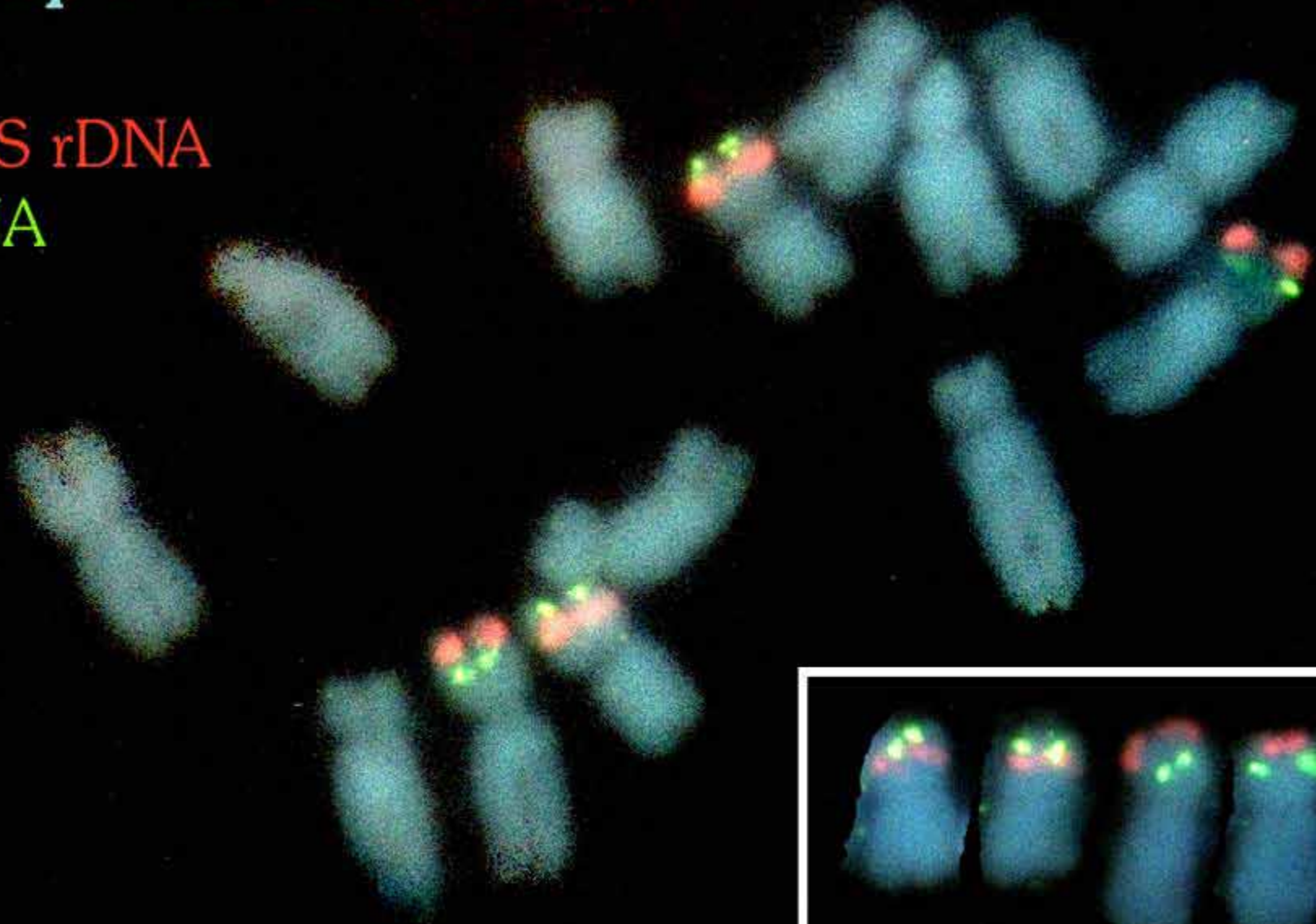
Chromosome
preparation

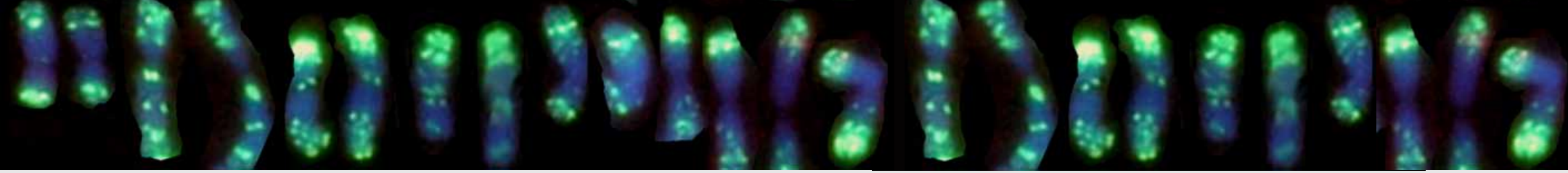
Aegilops umbellulata

DAPI

18S-25S rDNA

5S rDNA



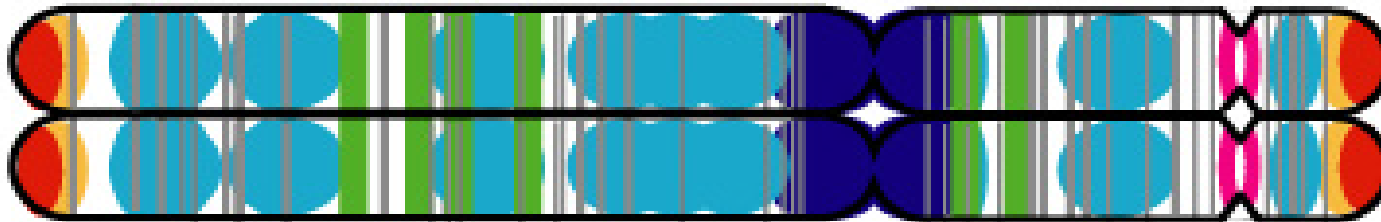


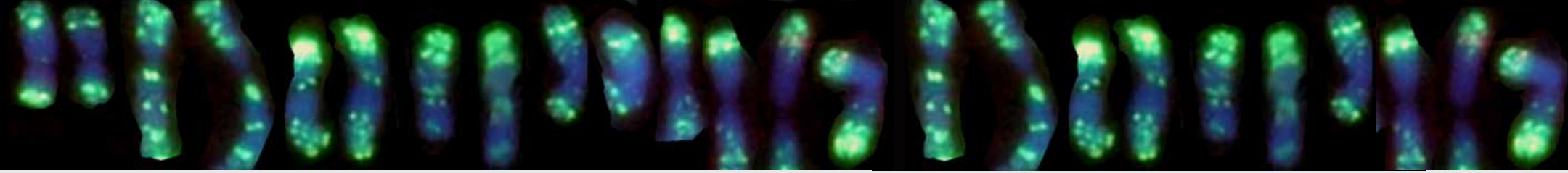
NOR: rDNA loci vary in number,
position and size



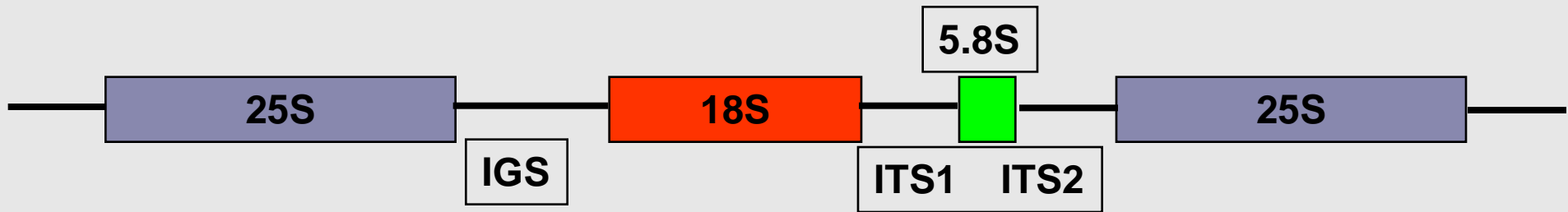
rDNA repeats

Chromosomal
Satellite



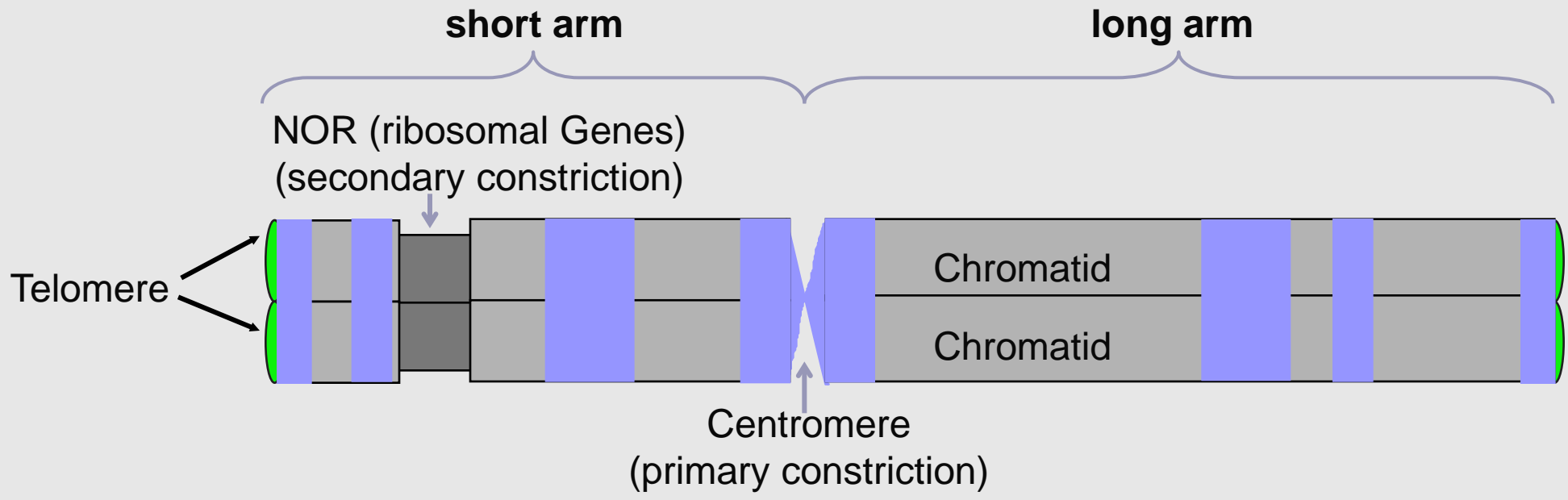


rDNA



- ☞ 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

Metaphase Chromosomes: structural components and terminology



Chromosome regions:

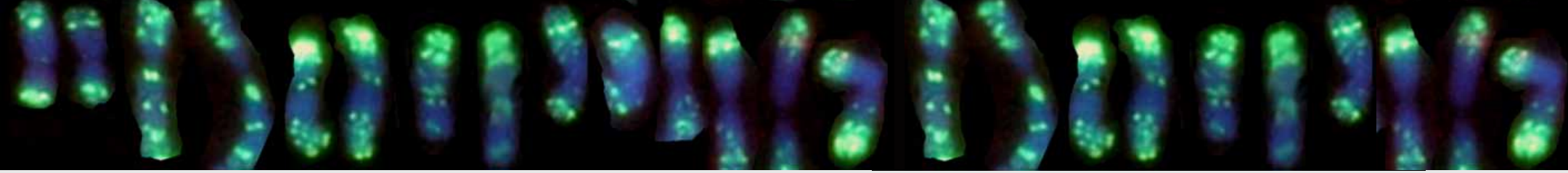
pericentric
(pericentromeric)

intercalar
(interstitial)

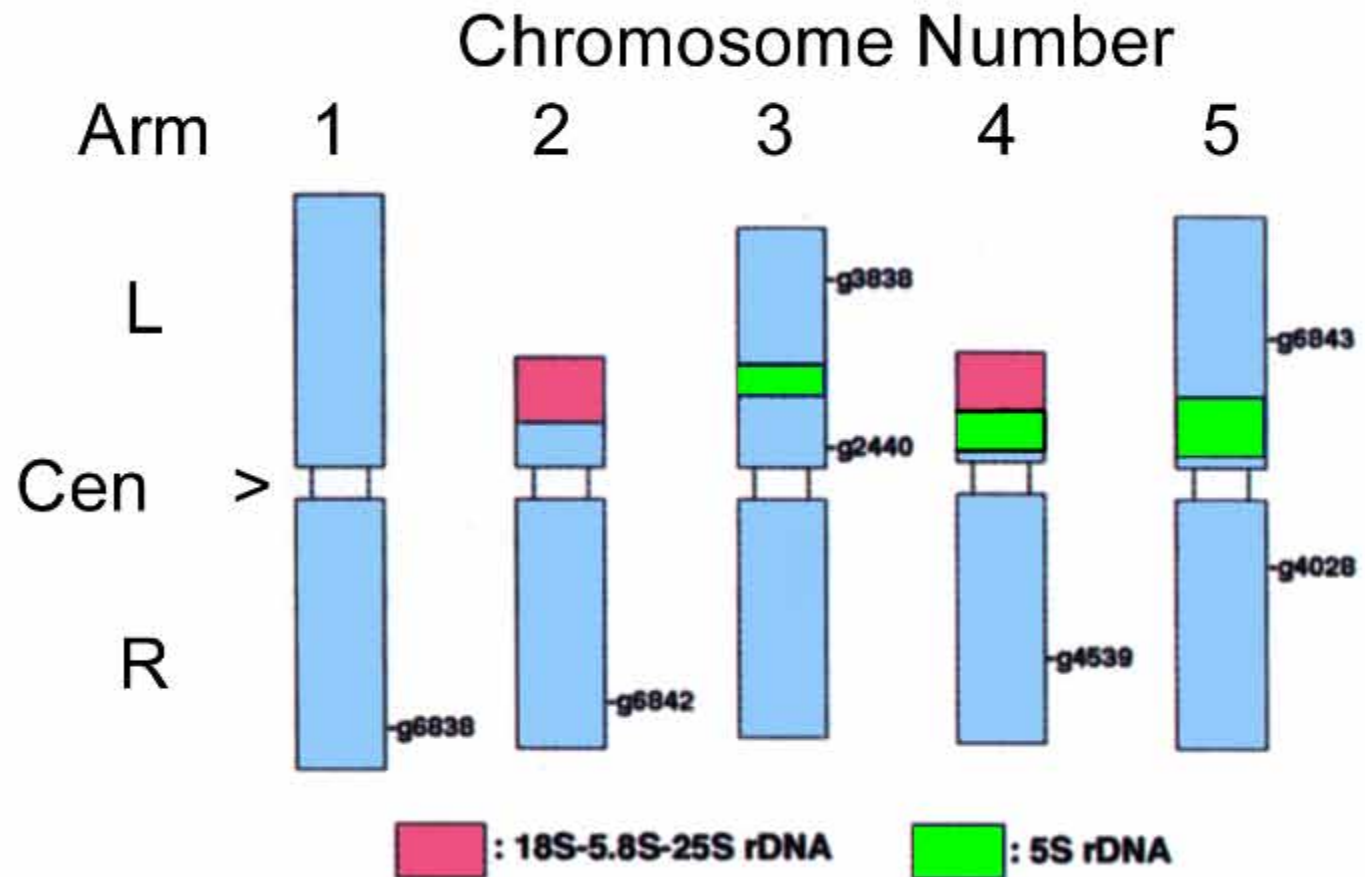
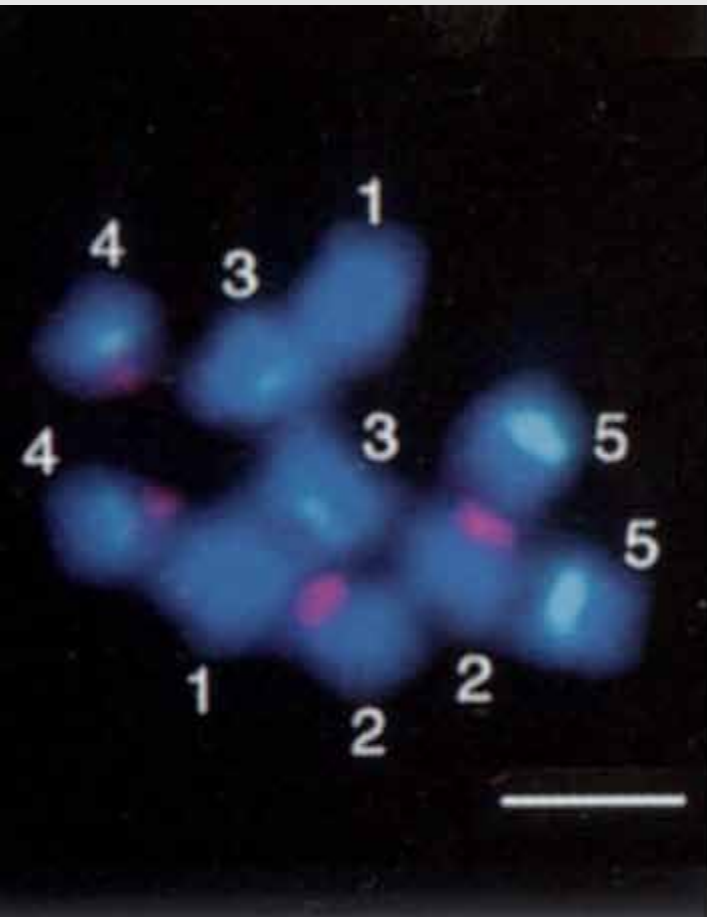
subtelomeric
(terminal)

- euchromatin, many genes
- heterochromatin, few genes

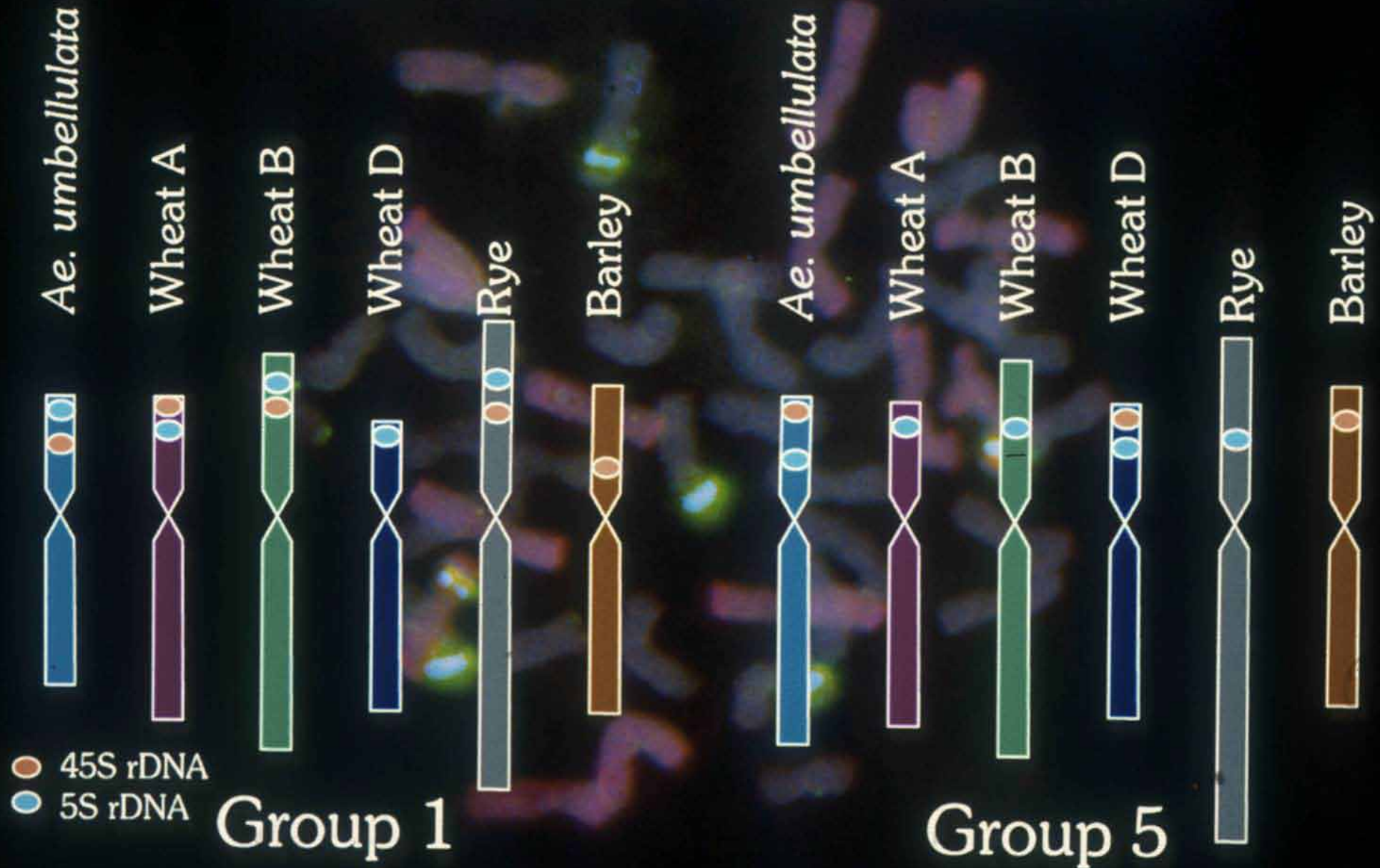
NOR: nucleolus organising regions

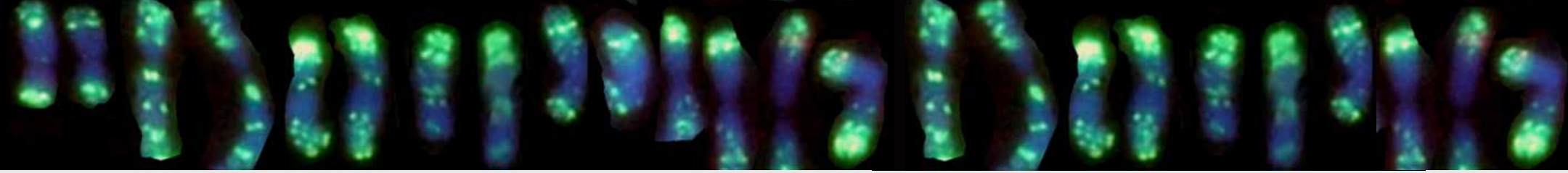


Arabidopsis thaliana $2n=10$



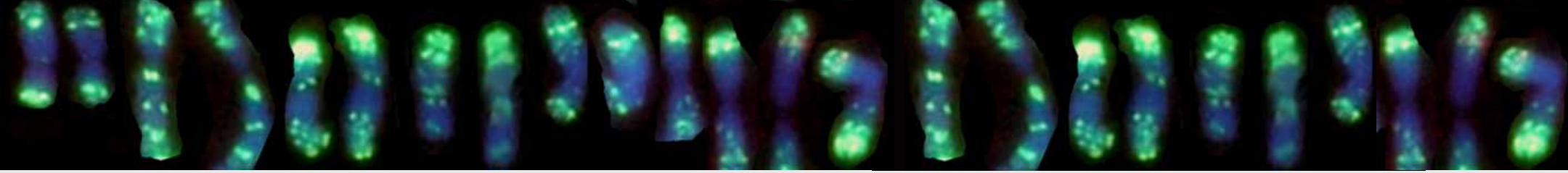
rDNA sites in Triticeae Genomes





Genome

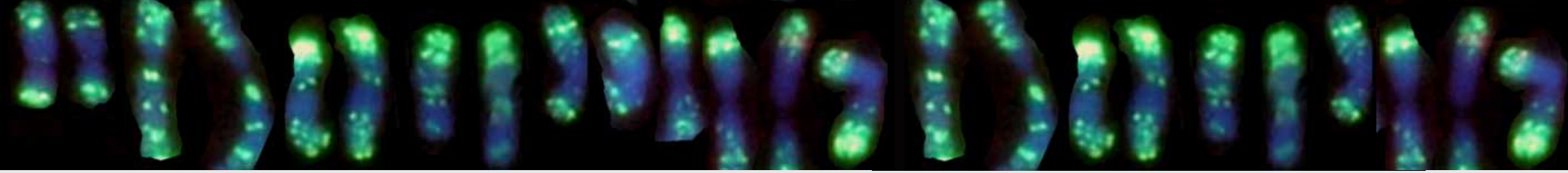
- ☛ Genes and regulatory sequences make up a small proportion of the genome
- ☛ The majority of DNA sequences in all higher eukaryotic genomes are repetitive sequences (50-90%)
- ☛ JUNK?



What is the rest of the genome?

☞ Repetitive DNA

- Repeated genes
 - rDNA (45S and 5S)
- Tandemly repeated, non-coding DNA



Repeated DNA sequences

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

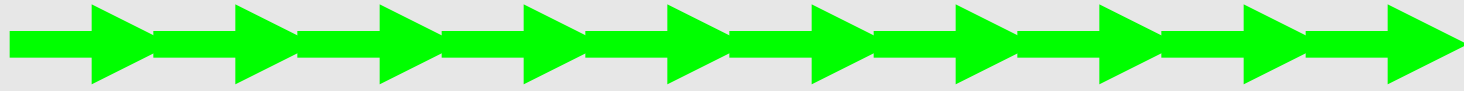
☞ Tandem repeats

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

Satellite: shoulder on CsCl gradient graphs



Tandem Repeats



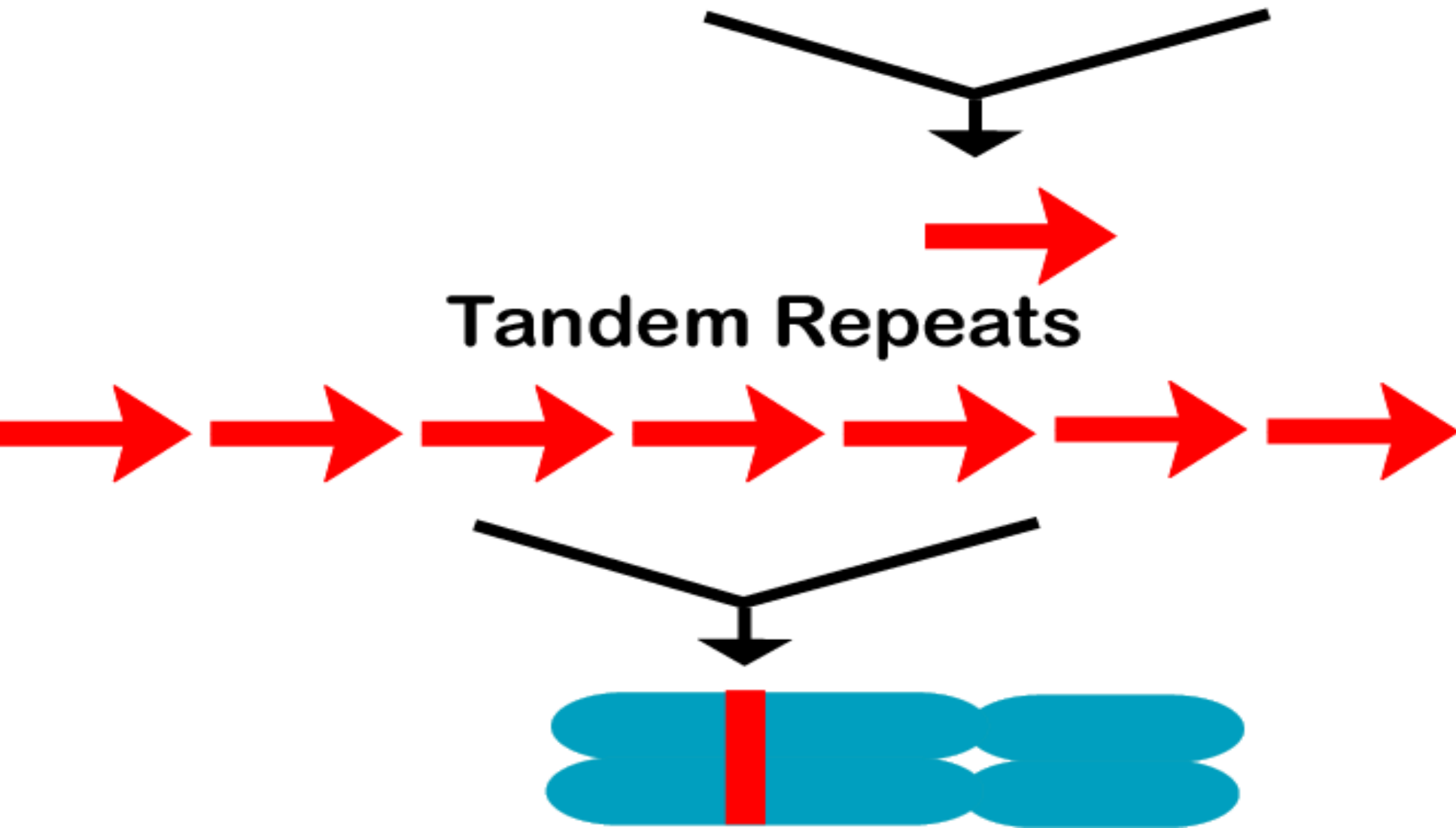
Where each arrow is a single unit of a repeat –
- often a multiple of 180 bp but up to 10kb long

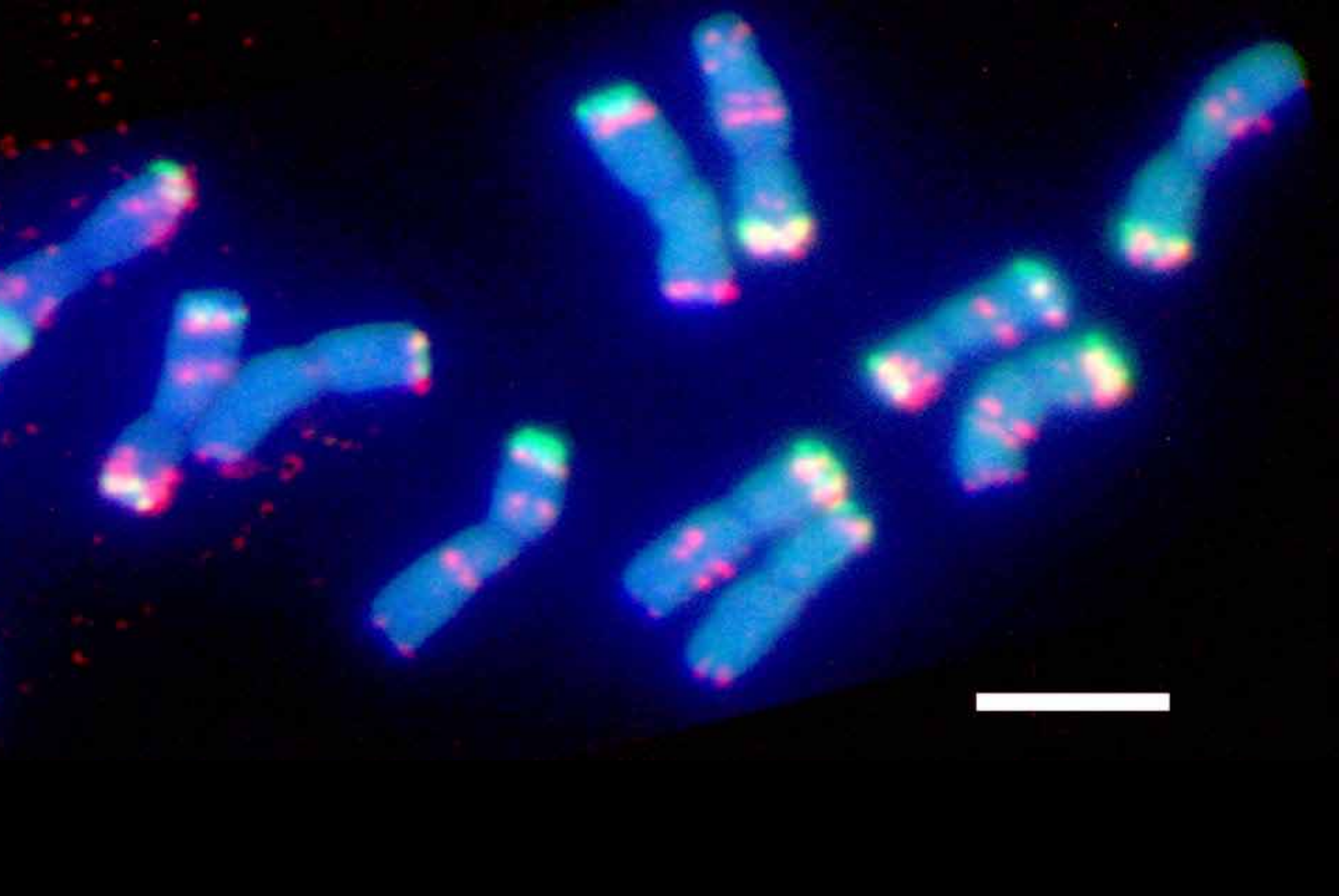
Head-to-tail organization

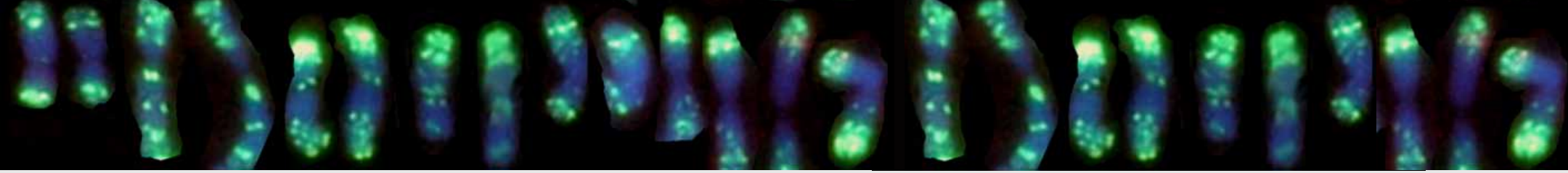
GCGCTAG GCGCTAG GCGCTAG GCGCTAG GCGCTAG GCGCTAG

Tandemly arranged DNA monomers

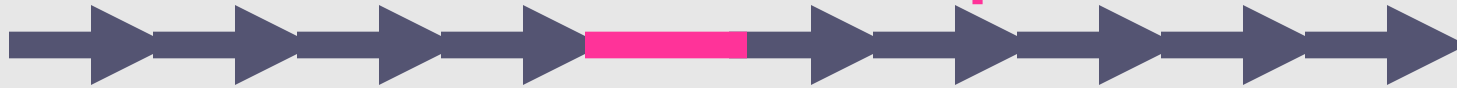
Repeat monomer: CCTAGCGTAACGGGTACGGGCTAGC



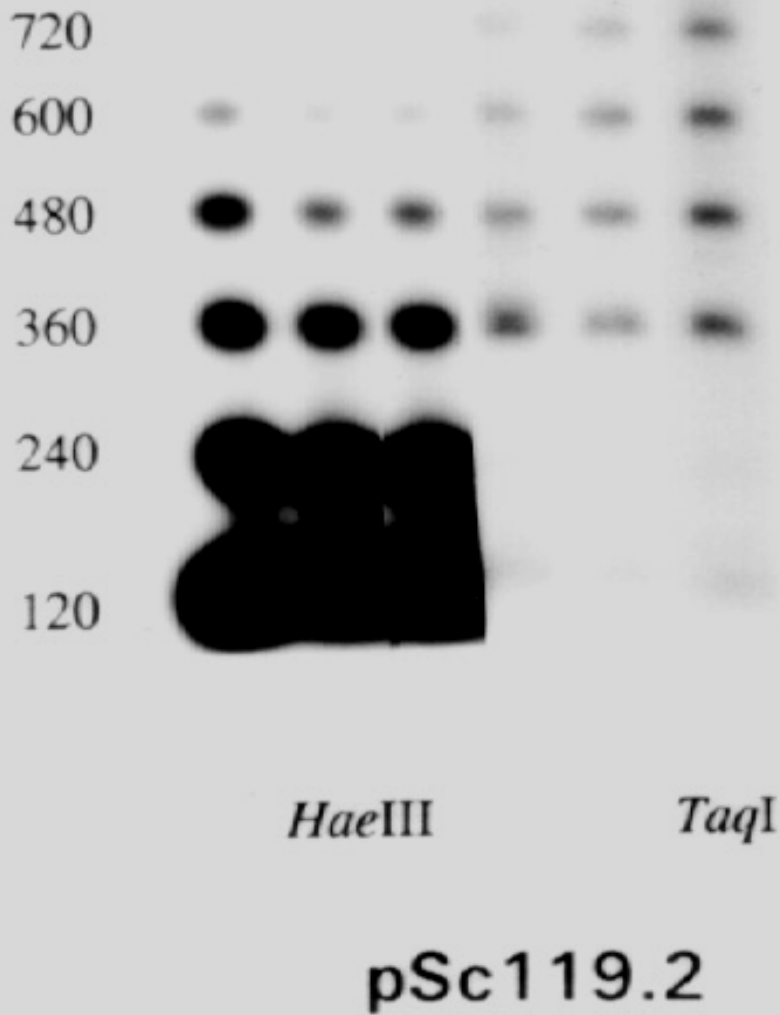




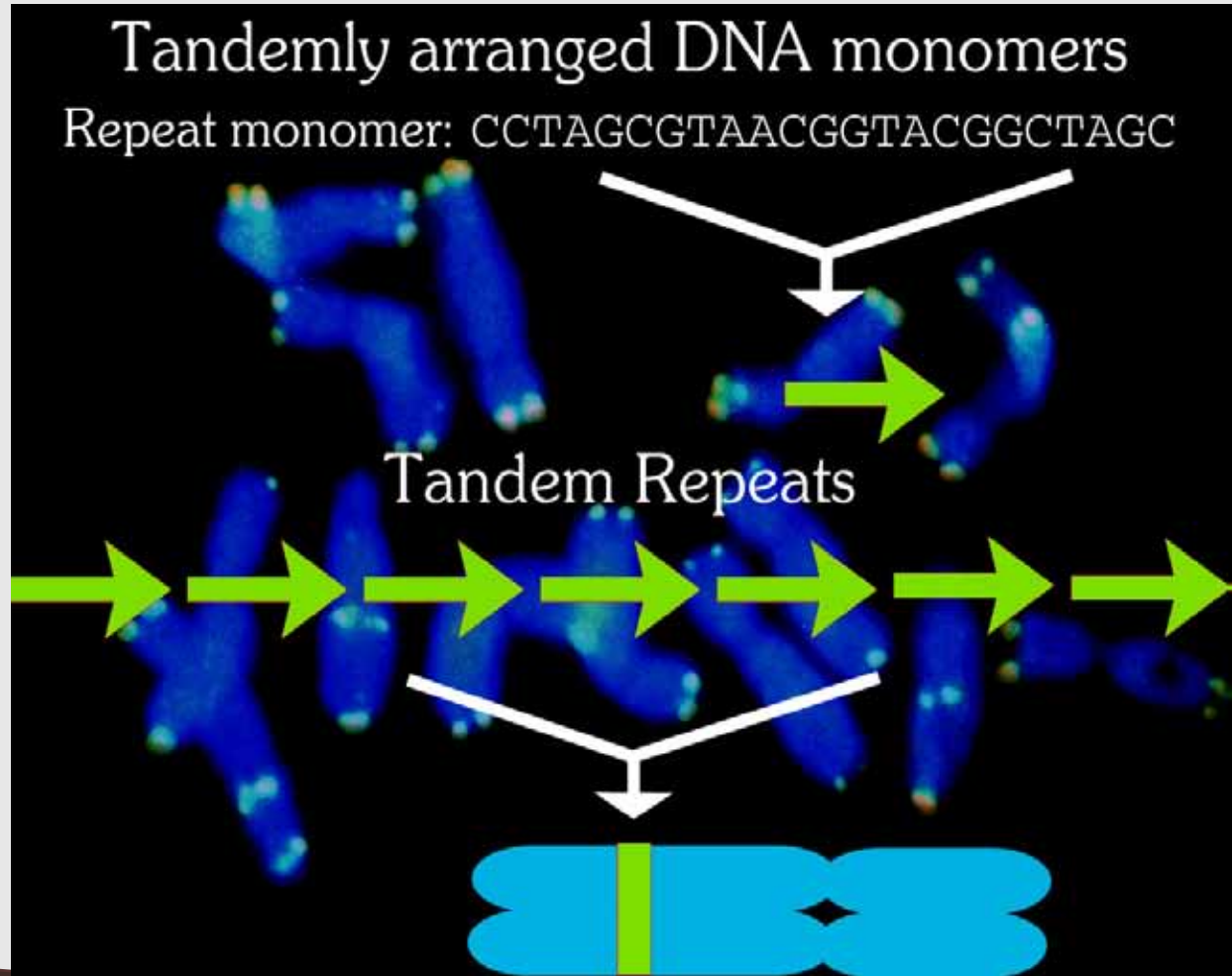
Tandem Repeats



- 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

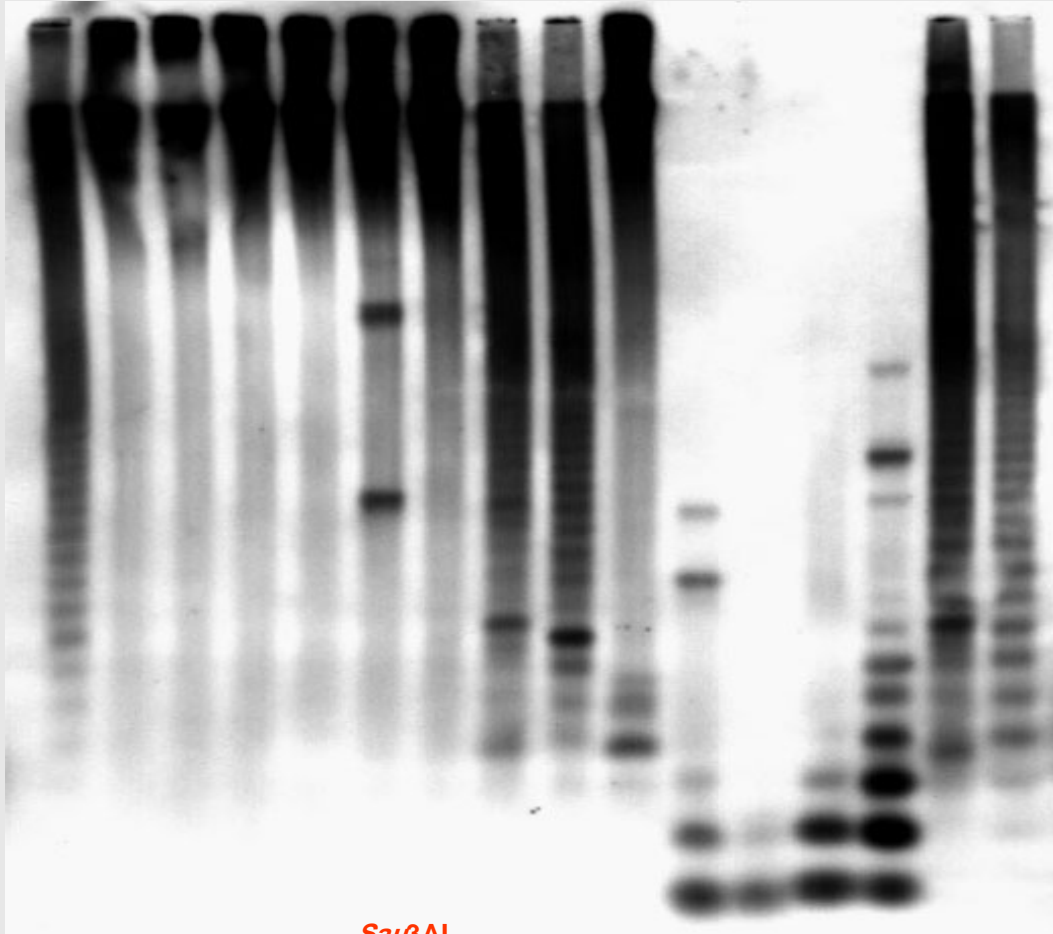


Tandem Repeats



Tandemly repeated DNA in Spruce

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



← 21,22 kb

← 5,14 kb

← 1584 bp

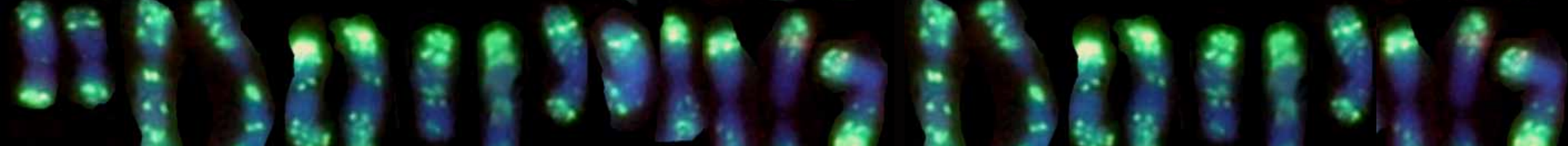
← 587 bp

← 267 bp

- 1=*MspI* mCCGG
- 2=*HpaII* mC^mCGG
- 3=*PstI* mCTGCAG
- 4=*BamHI* GGAT^mCC
- 5=*SacI* GAG^mCTC
- 6=*HindIII* AAG^mCTT
- 7=*PvuII* CAG^mCTG
- 8=*AluI* AG^mCT
- 9=*TaqI* TCGA
- 10=*HaeIII* GG^mCC
- 11=*MseI* TTAA
- 12=*Tsp509I* AATT
- 13=*ApdI* PuAATTPy
- 14=*Sau3AI* GAT^mC
- 15=*DraI* TTTAAA
- 16=*EcoRV* GATATC

PATR140 consensus *Sau3AI* GATCTCGCCG TTTTTTTG-- ----TTTTT TTTTTT-TTT TTACAGCTGC GGGGTTATGC
 TTTCGT-CTG AAGAATGCTG CAGTGC--TG AAATCCCCCA AATTCTGAAC CAGAGAGTAT
 TTAAGTAGCA A-CCCGAATA CAGATGCGGG TTAATA *MseI* *Tsp509I* *PvuII*

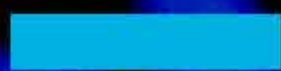
Morgante, Jurman, H



DNA packs around nucleosomes



Histone octamer

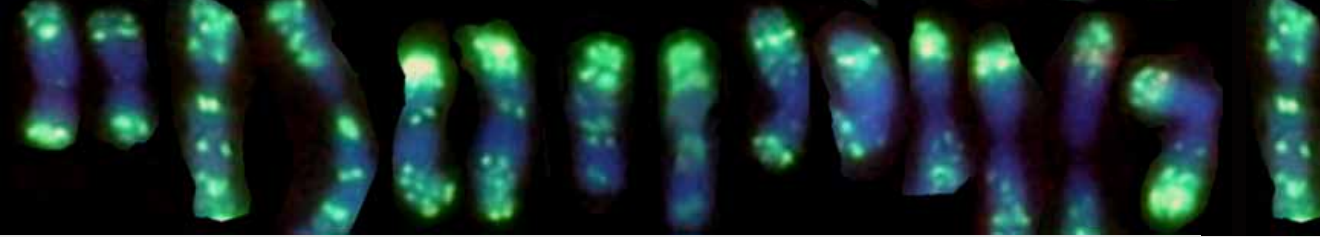


DNA double helix



Linker
(variable bp)

Two full turns (170 bp)

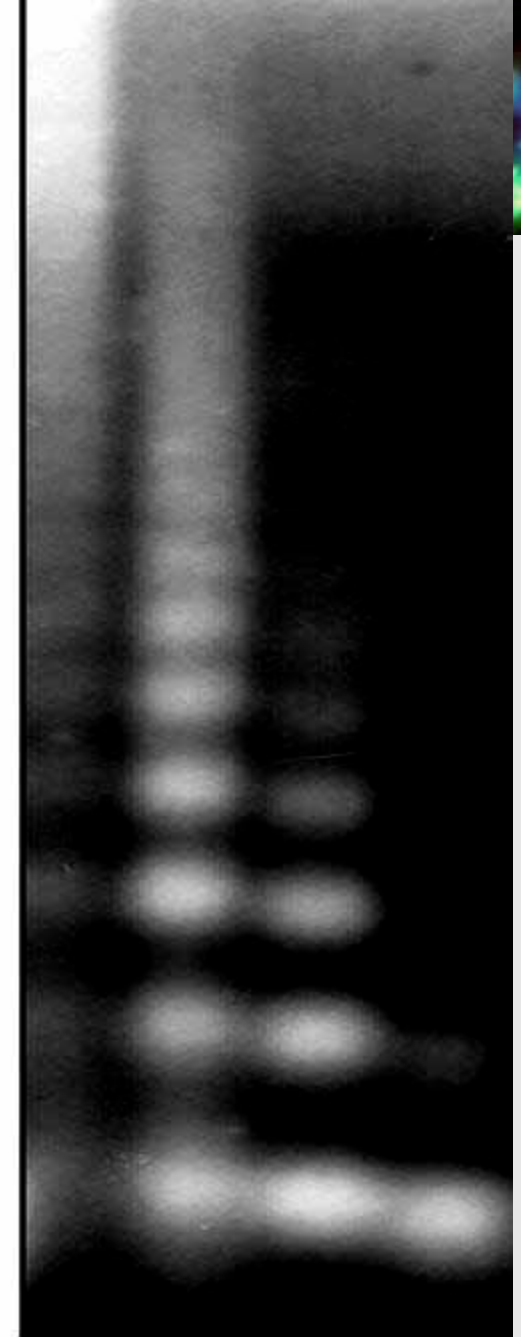


Nucleosomes

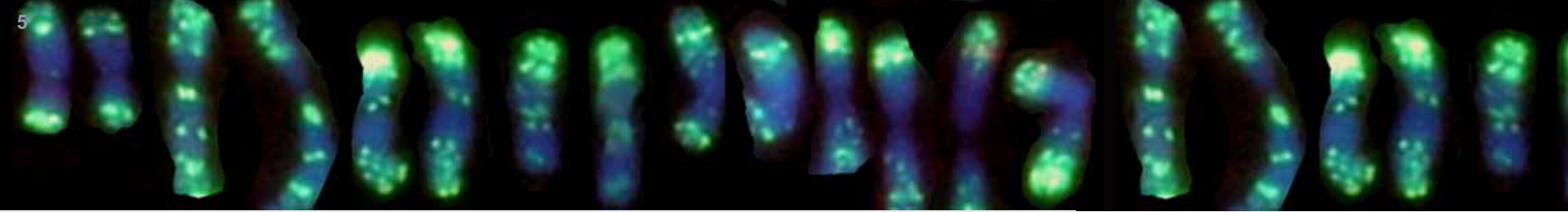
Digest intact chromatin (DNA + histone) with micrococcal nuclease for a few seconds, cutting between the nucleosomes. Then treat with protease and run on agarose gel.

- ↳ Vershinin &
- ↳ Heslop-Harrison

bp
680
510
340
170



15
45
90
270
seconds



Isolation of satellite-DNA by restriction enzyme analysis (Restriction satellites)

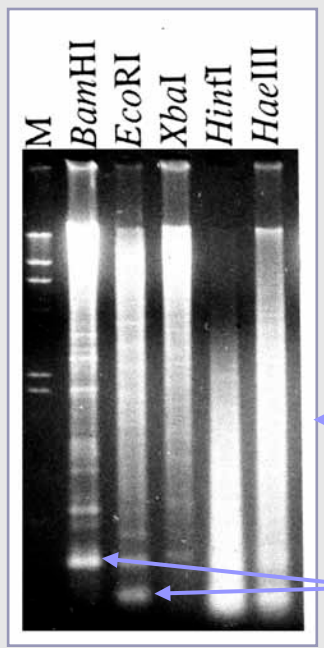
Example: *Beta vulgaris*, sugar beet

1. Isolation of genomic DNA (Species A)

2. Restriction enzyme digest and gel electrophoresis

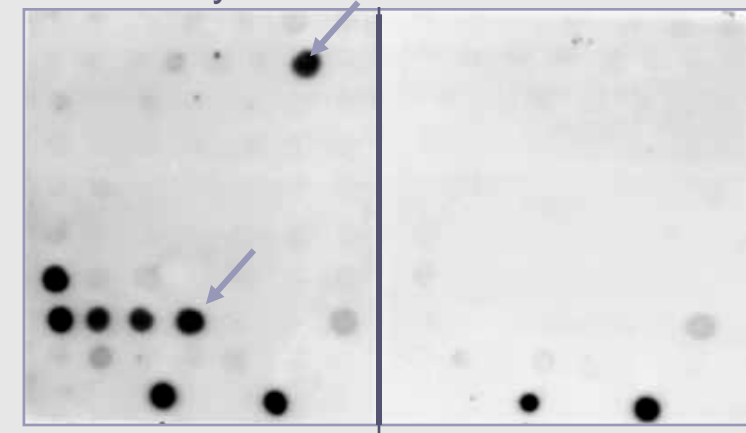
3. Elution and cloning of the repeats in plasmids

4. Selection of plasmid clones by Dotblot-Hybridisation



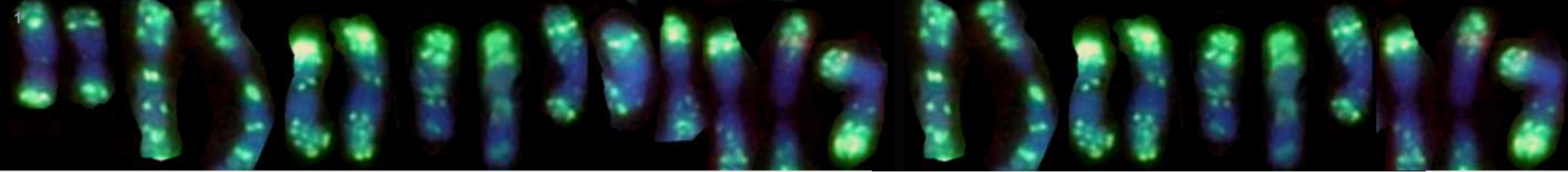
} Restricted genomic DNA

Conserved Restriction fragment (Restriction satellite)



Species A Species B
Probe: genomic DNA

5. Molecular analysis Sequencing



Molecular Analysis of satellite-DNA

Sequencing:

- Monomers (basic repeat unit)



Variability

(Divergence of the satellite-DNA)

GGATCCGTCGT

GGATTCGTCGA

GATTTC-TCGA

G GATCCGTCGT

G GATTCGTCGA

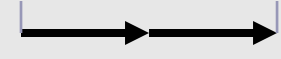
T CATTGGTCAG

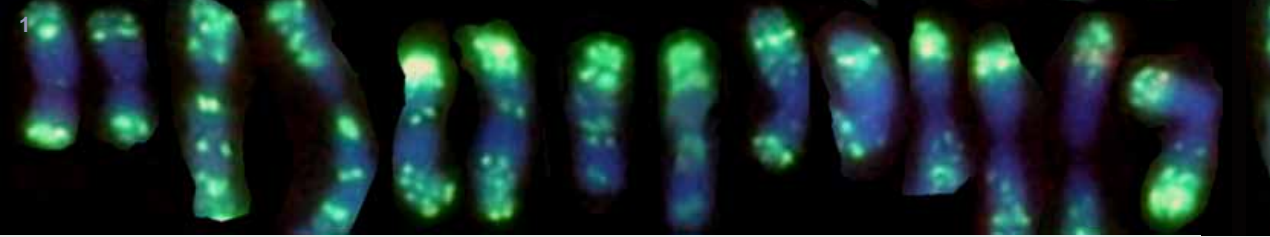
Genome organisation:

- Southern hybridisation



Physical localisation



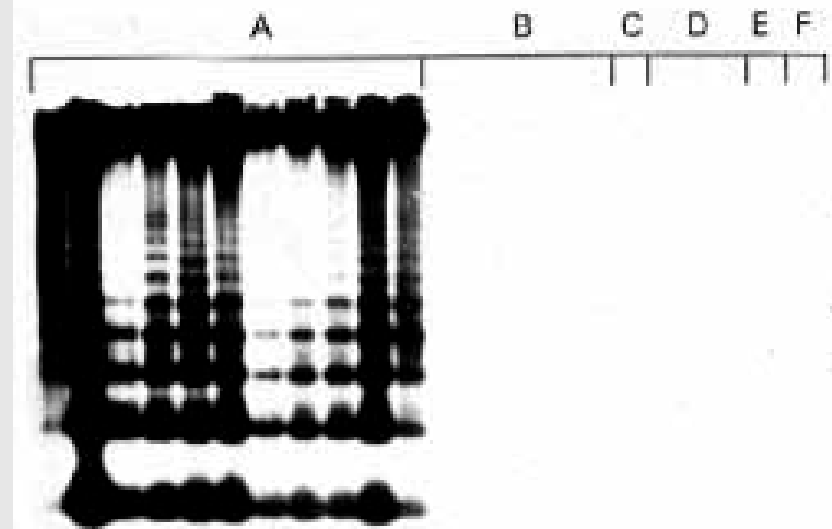
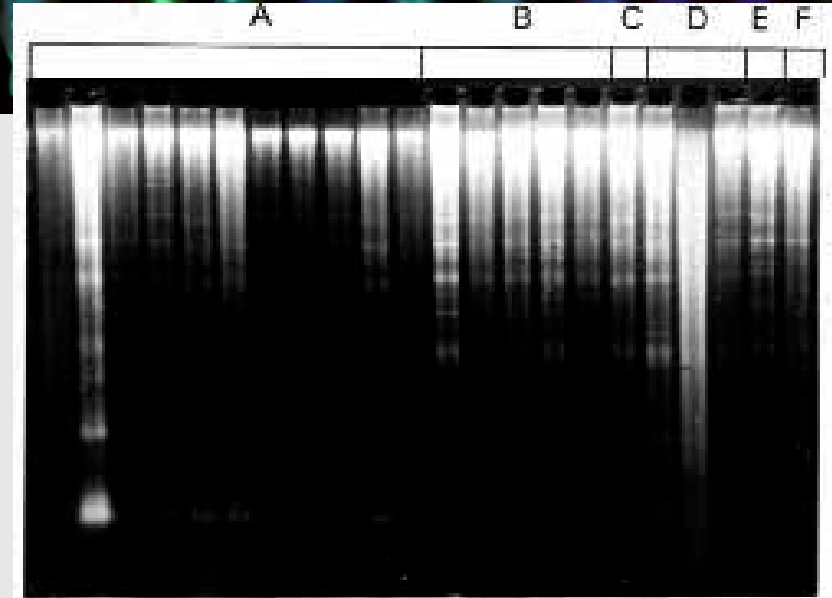
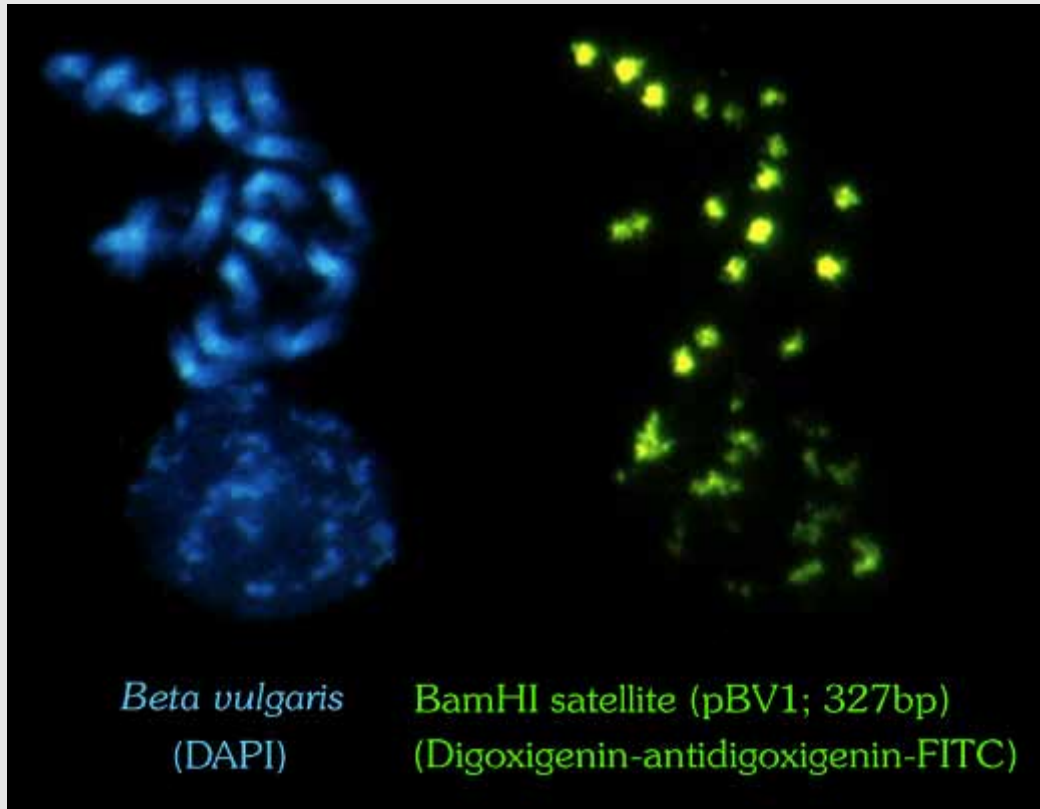


Phylogeny and evolution:

- Southern analysis of related species

Analysis of repeated DNA sequences on many different levels

- Fluorescent-*in situ*-Hybridisation (FISH)

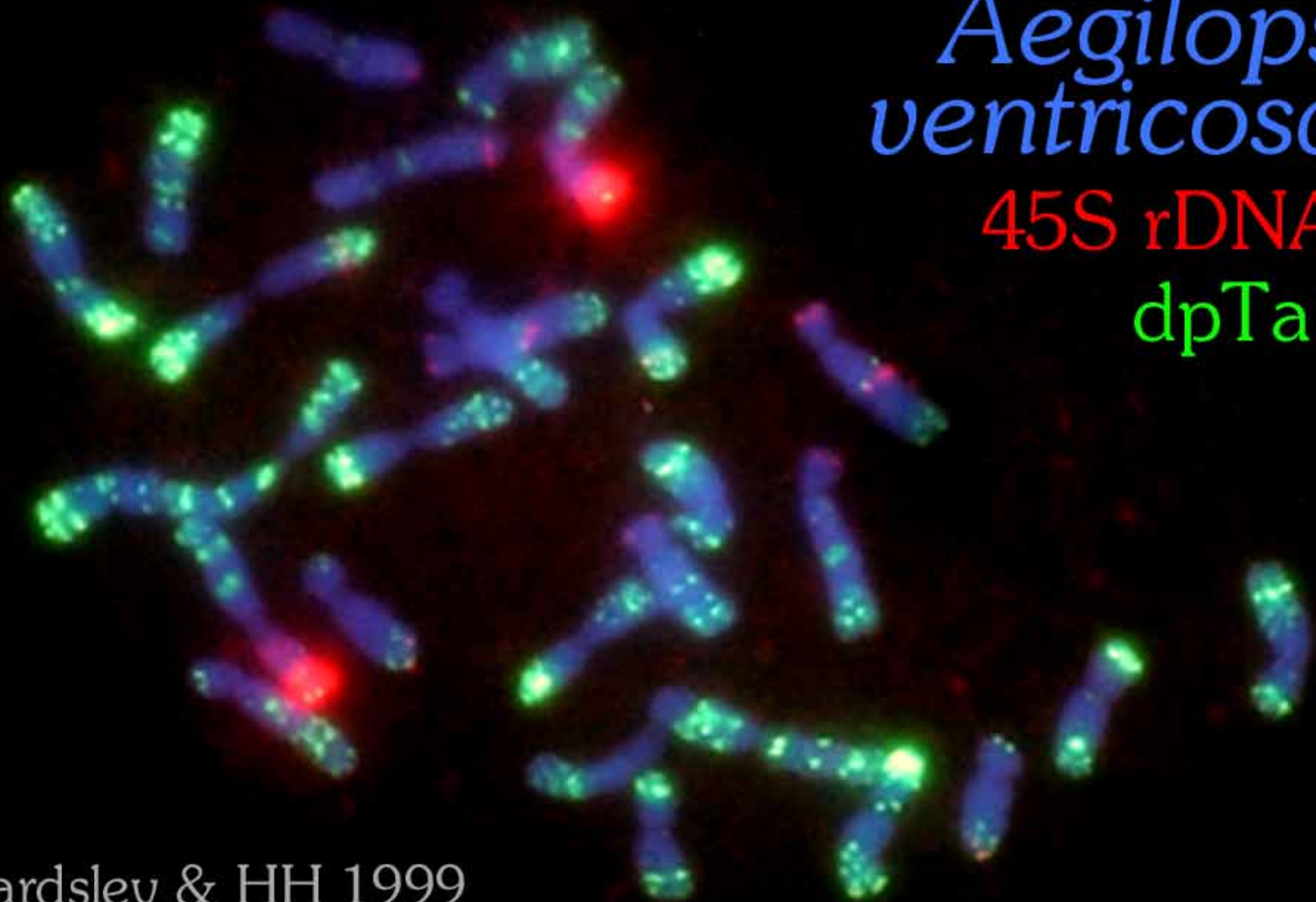


A: Section *Beta* B: Section *Corollinae*
 C: Section *Nanae* D: Section *Procumbentes*
 E: *Spinacea oleracea* F: *Chenopodium b.-hennicus*

*Aegilops
ventricosa*

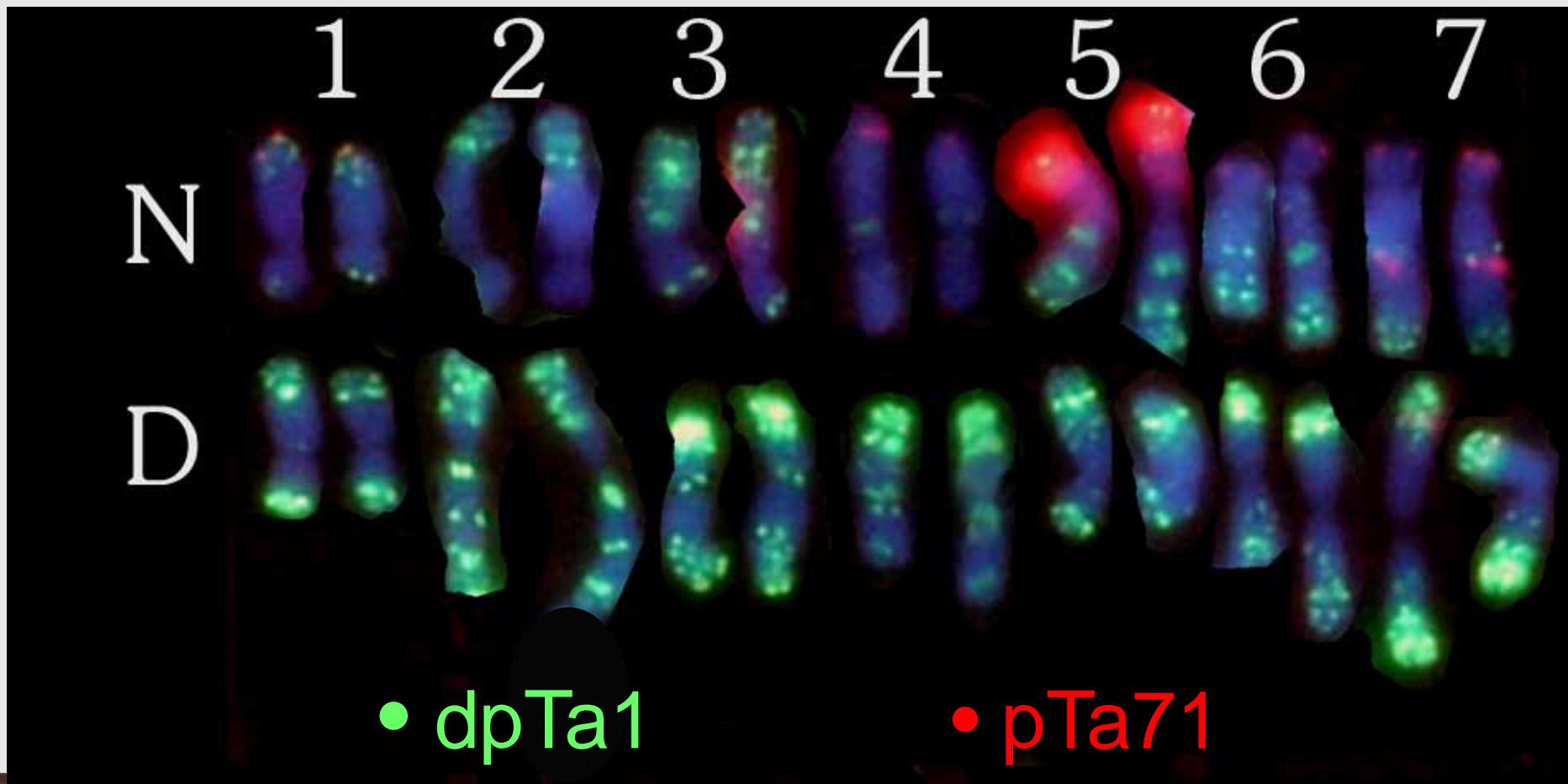
45S rDNA

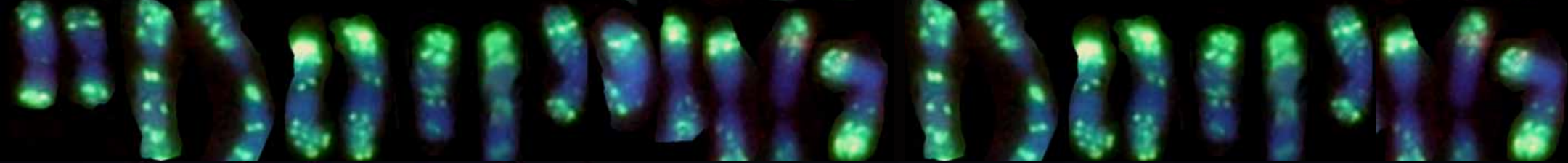
dpTa1



Differences between genomes

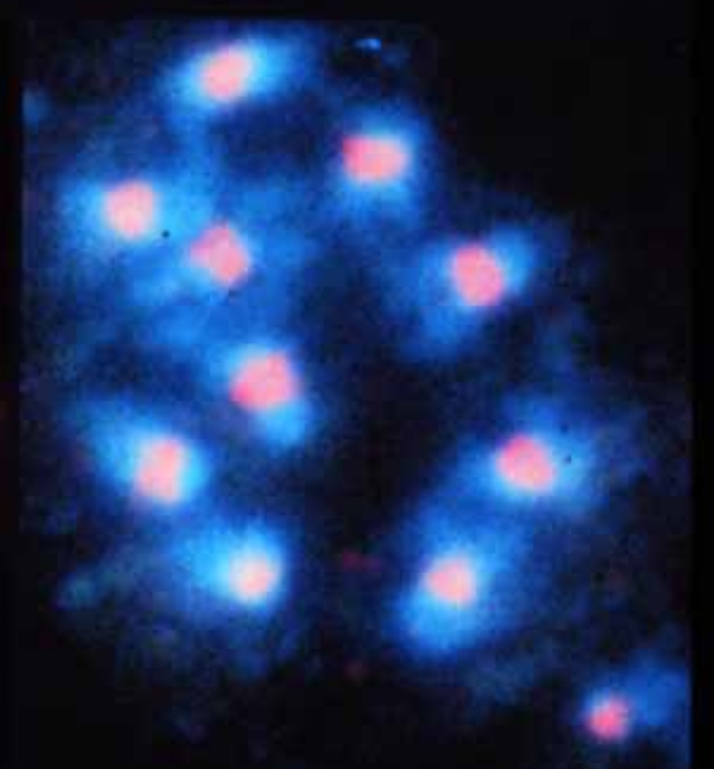
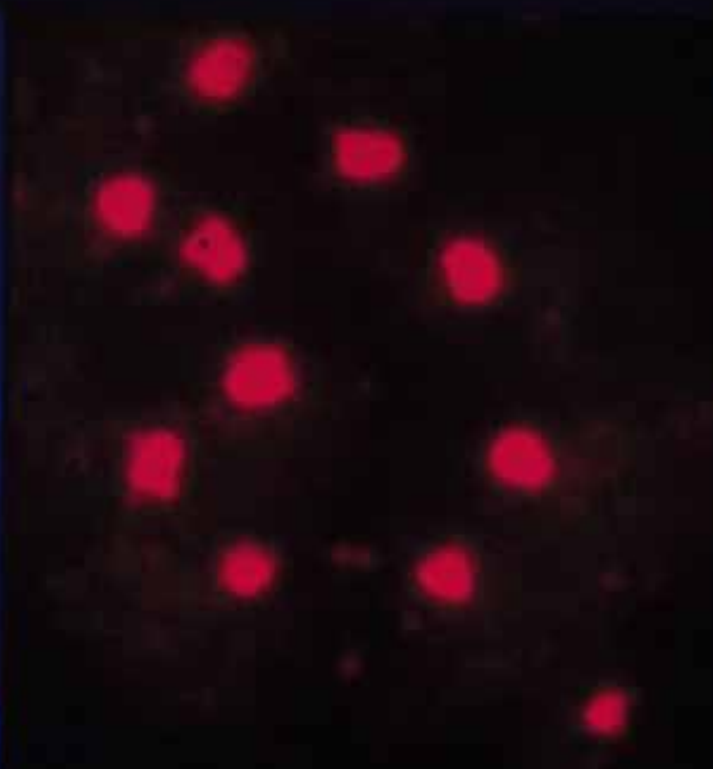
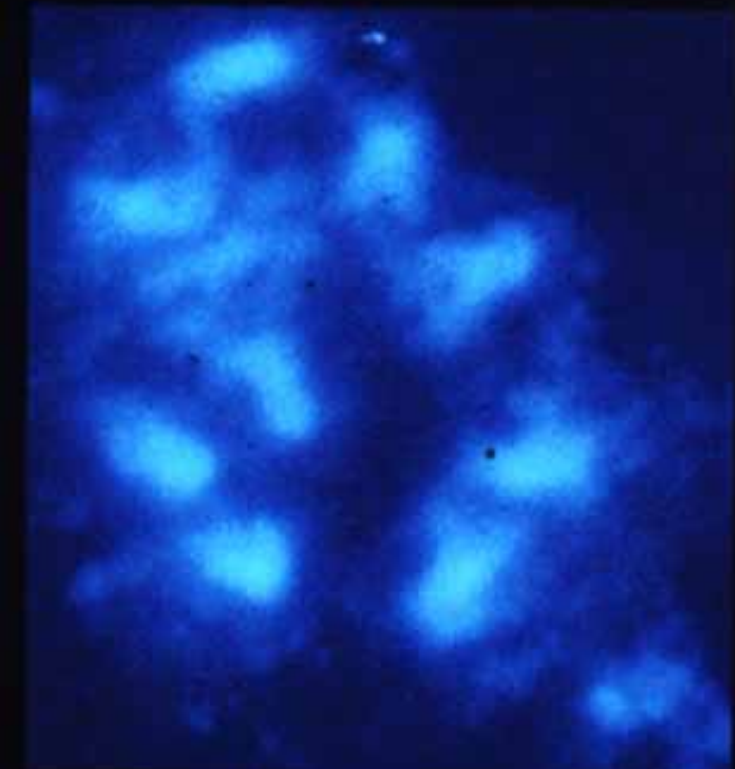
Major differences in the nature and amount of repetitive DNA

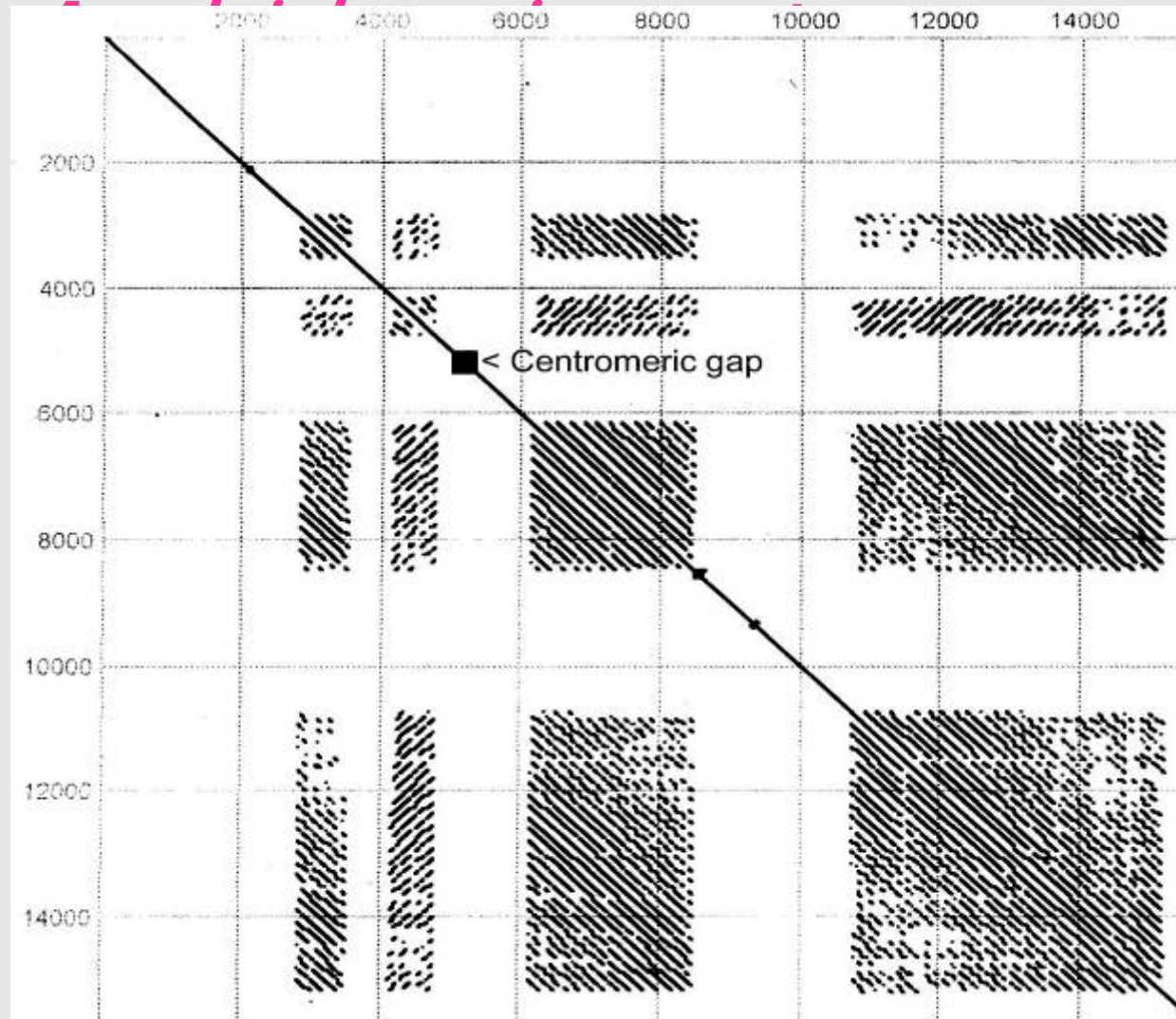
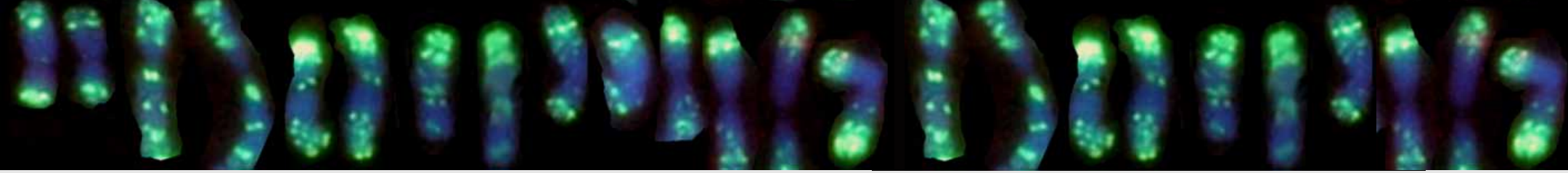




Arabidopsis thaliana

In situ hybridization with AtCen

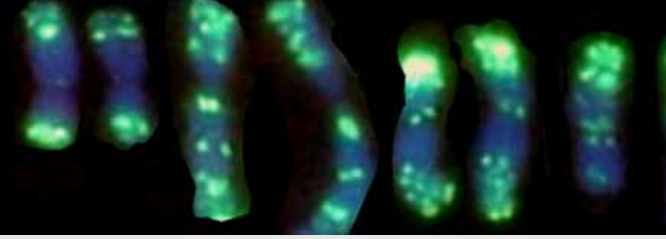




	←Primer site I→				Box A	←Primer site II →			
	1	2	3	4	5	6	7	8	
	123456789012345678901234567890123456789012345678901234567890123456789								
AtHR220/2	1: AACCTTCTTCTTGCTTCTCAAAGCTTTGATGGTTAGCCGAAGTCCATATGAGTCTTTCTCTTTGTATCTTCTAACAAG-GAAACACTAC								
179:	G.....		GTAT..I.....	G.....		AT.....	A.....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

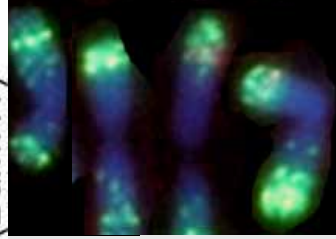
AtHR220/2	1: AACCTTCTTCTTGCTTCTCAAAGCTTTGATGGTTAGCCGAAGTCCATATGAGTCTTTGTCTTTGTATCTTCTAACAAG-GAAACACTAC
Aa27	80:A.....TT...GTG...G.G..G.....-.....T..CGGT...GAG.....ACG.AA...T.....
Aa271	80:A.....T...GTG...G.G..G.....-.....T..CGGT...GAG.....ACG.AA.....
Aa214	80: T-----C.....-TG.TCCCT..C.....-.....T..CGGT...GAG...-...ACG.A.....
Aa519	80: T-----T...C.-TG...A-G..-.....-.....T..CGGT...GAG.....ACG.AA.....G
Aa524	80: ..G.....A.....-----T..CAACG...-G..-.....-.....T..CGGT...GAG.....ACG.AA.....C.

	←Primer site III →			Box B					
	1	1	1	1	1	1	1		
	9	0	1	2	3	4	5	6	7
AtHR220/2	0123456789012345678901234567890								
	TTAGGCTTGTAGGAGAAGATTGCGGTTTAAG			TTCTTATACTCAATCATA					
T..G...TAA.G...A.....		C...C.....					
T..T..G...TAA.G.....		C...C.....					T.....
AtHR220/3T.....T..G...TAA.G.....		T...C...C.....					
T.....T..G...TAA.A..T.....		C...C.....					
T.....T..G...TAA.G.....		T...C...C.....					
G...TAA.A.....		C...C.....					



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

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



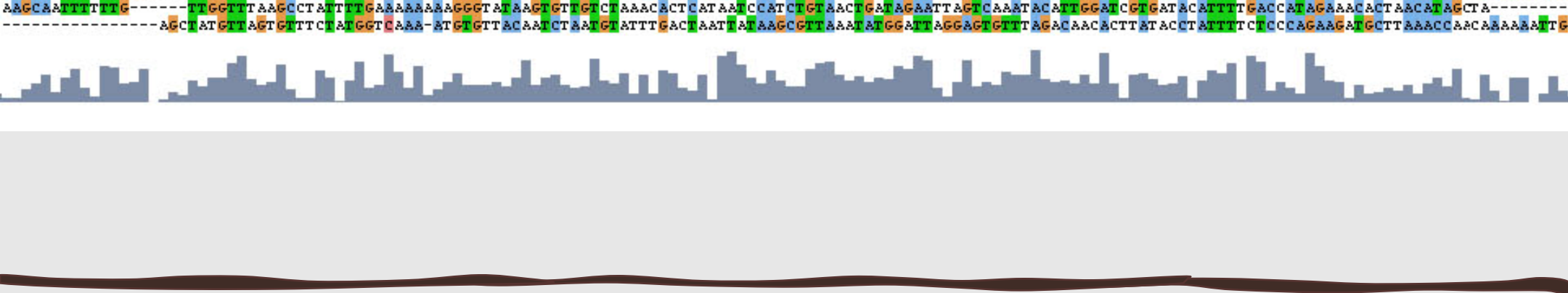
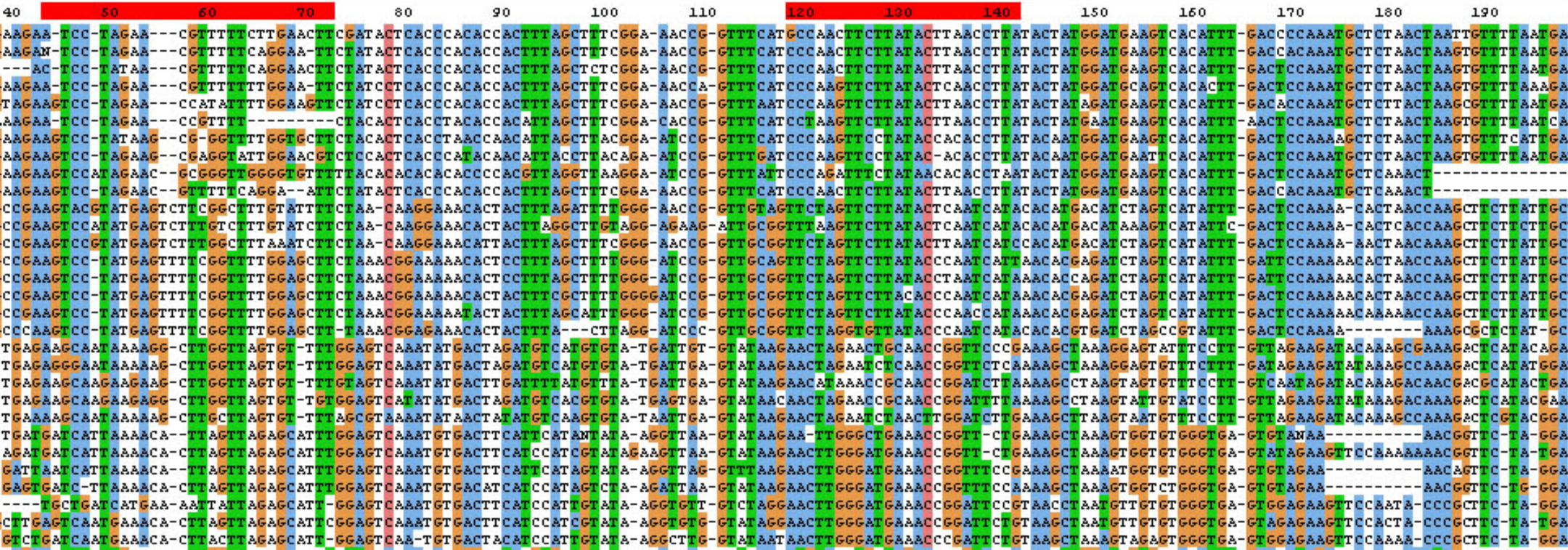
A. griffithiana
 (BamH1)

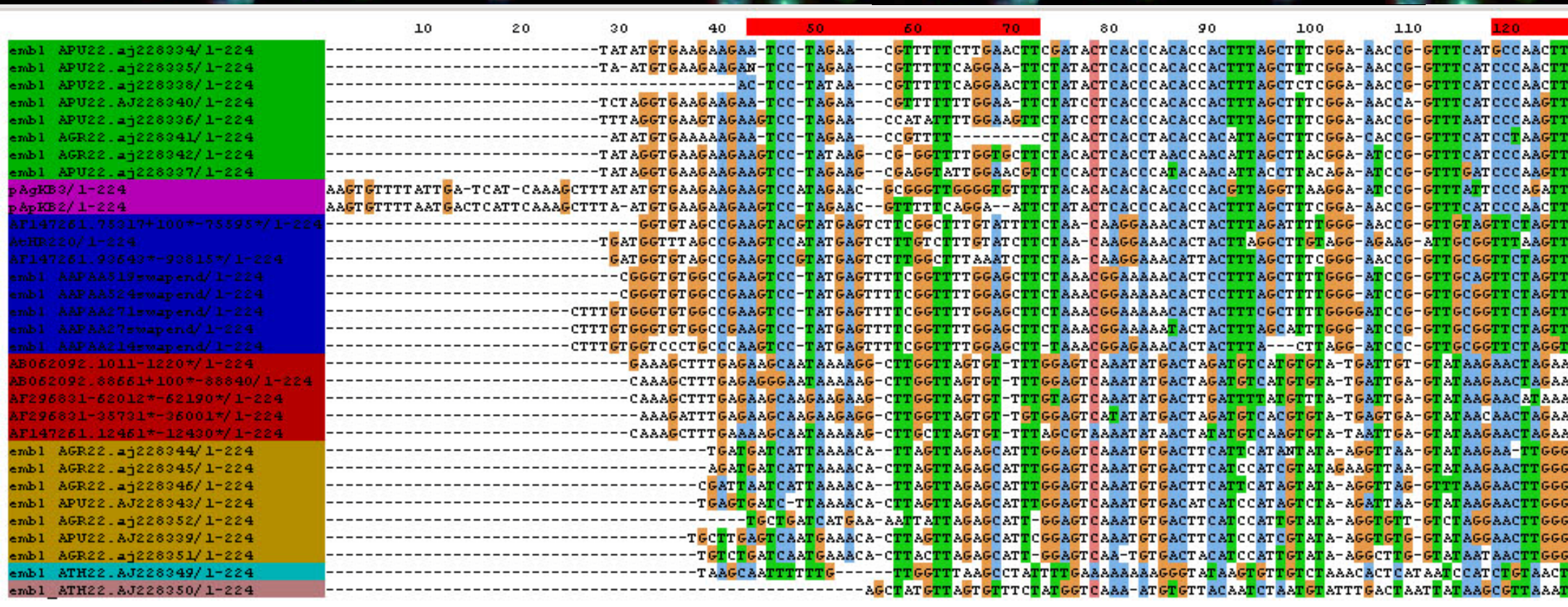
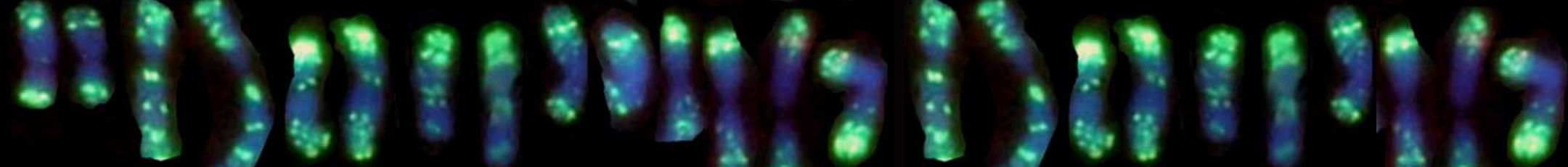


HaellI digests; Probe origin
A. griffithiana *A. pumila* *A. grif*

HH, Brandes
 2003

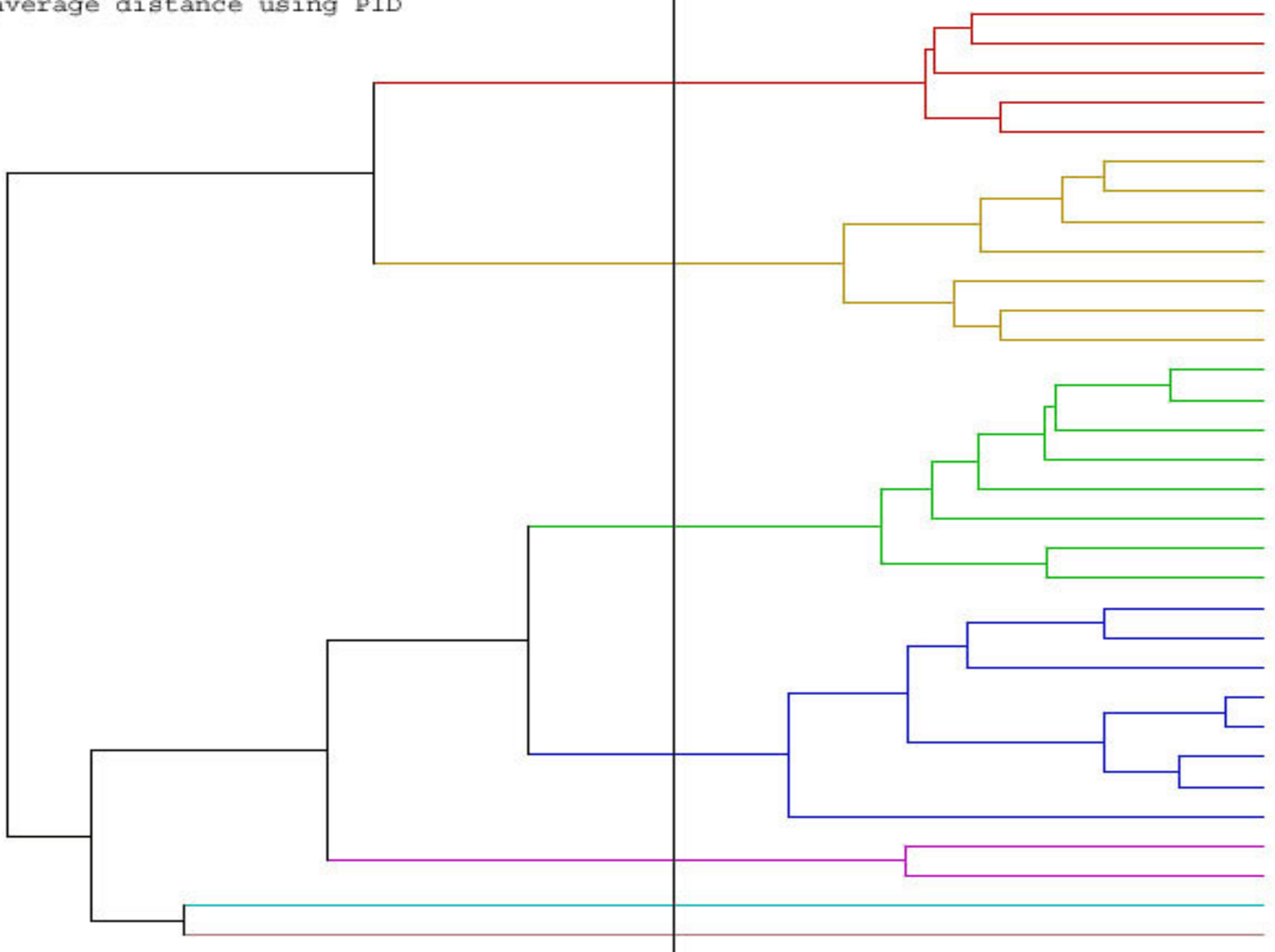
180bp in five *Arabidopsis* species







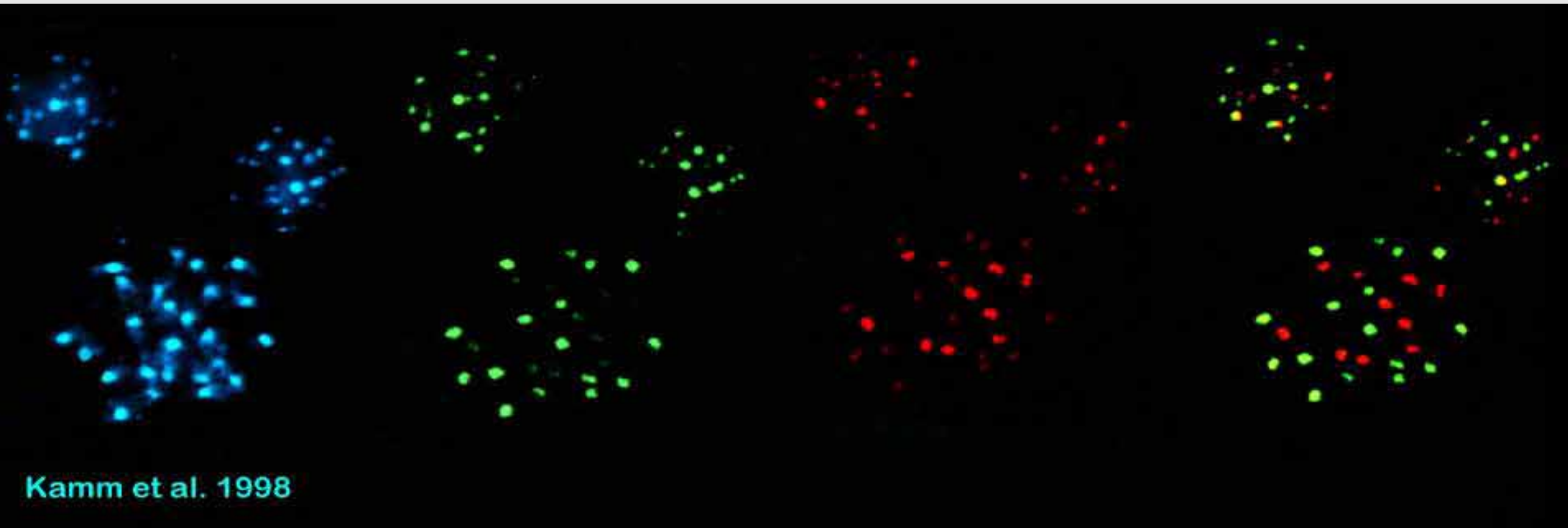
average distance using PID



- AB062092.1011-1220*
- AB062092.88661+100*-888
- AF147261.12461*-12430*
- AF296831-62012*-62190*
- AF296831-35731*-36001*
- embl_AGR22.aj228344
- embl_AGR22.aj228346
- embl_APU22.AJ228343
- embl_AGR22.aj228345
- embl_AGR22.aj228352
- embl_APU22.AJ228339
- embl_AGR22.aj228351
- embl_APU22.aj228334
- embl_APU22.aj228335
- embl_APU22.AJ228340
- embl_APU22.aj228336
- embl_APU22.aj228338
- embl_AGR22.aj228341
- embl_AGR22.aj228342
- embl_APU22.aj228337
- AF147261.75317+100*-755
- AF147261.93643*-93815*
- AtHR220
- embl_AAPAA519swapend
- embl_AAPAA524swapend
- embl_AAPAA271swapend
- embl_AAPAA27swapend
- embl_AAPAA214swapend
- pAgKB3
- pApKB2
- embl_ATH22.AJ228349
- embl_ATH22.AJ228350

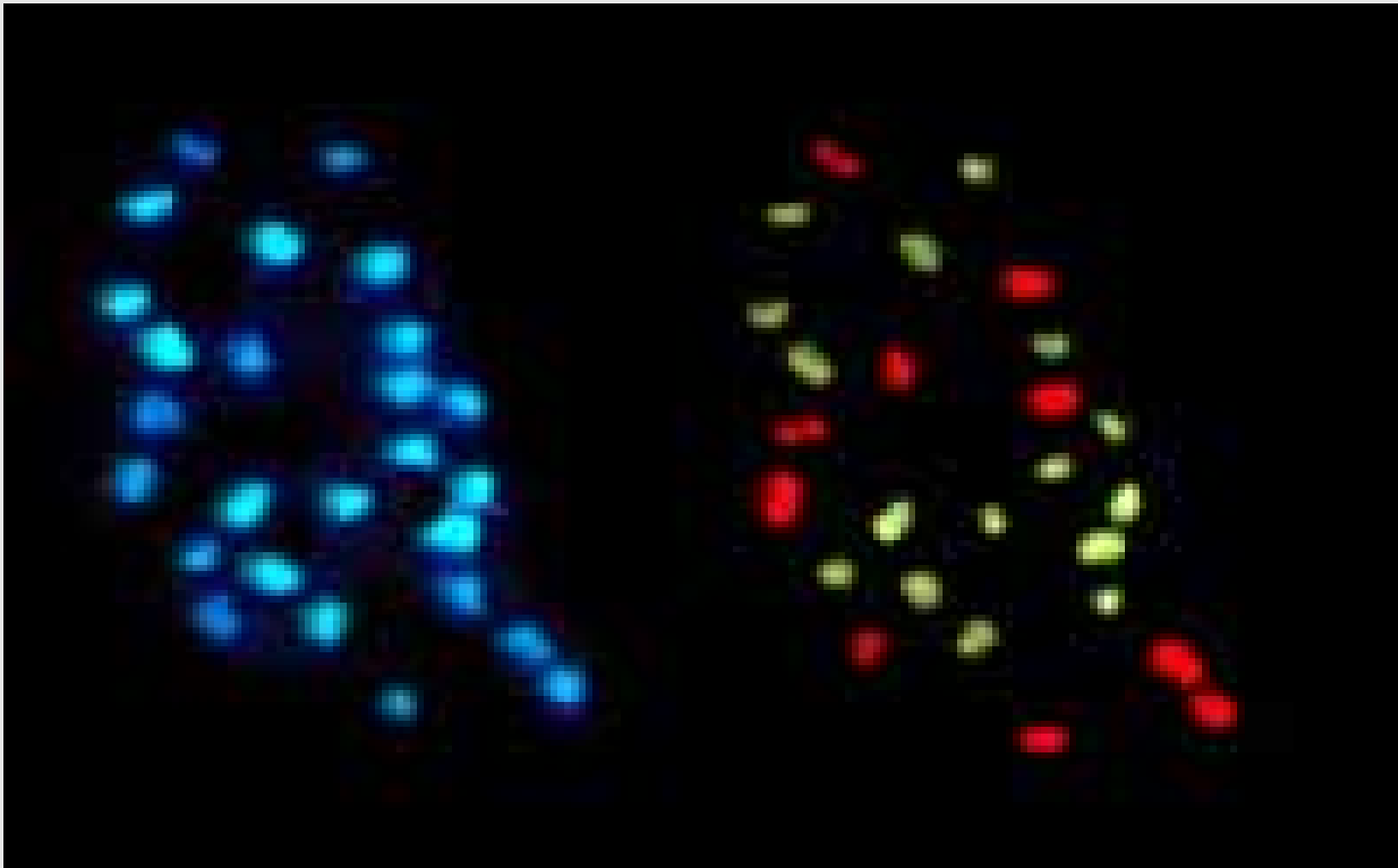
Arabidopsis suecica

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



Arabidopsis suecica

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



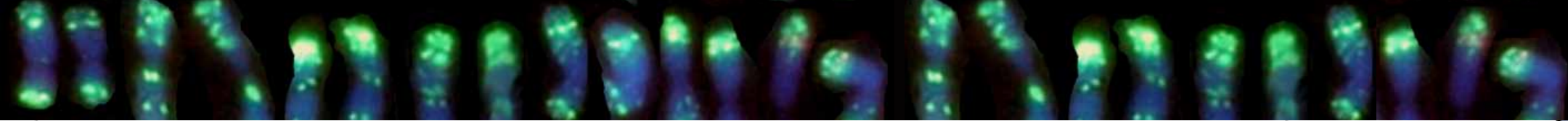
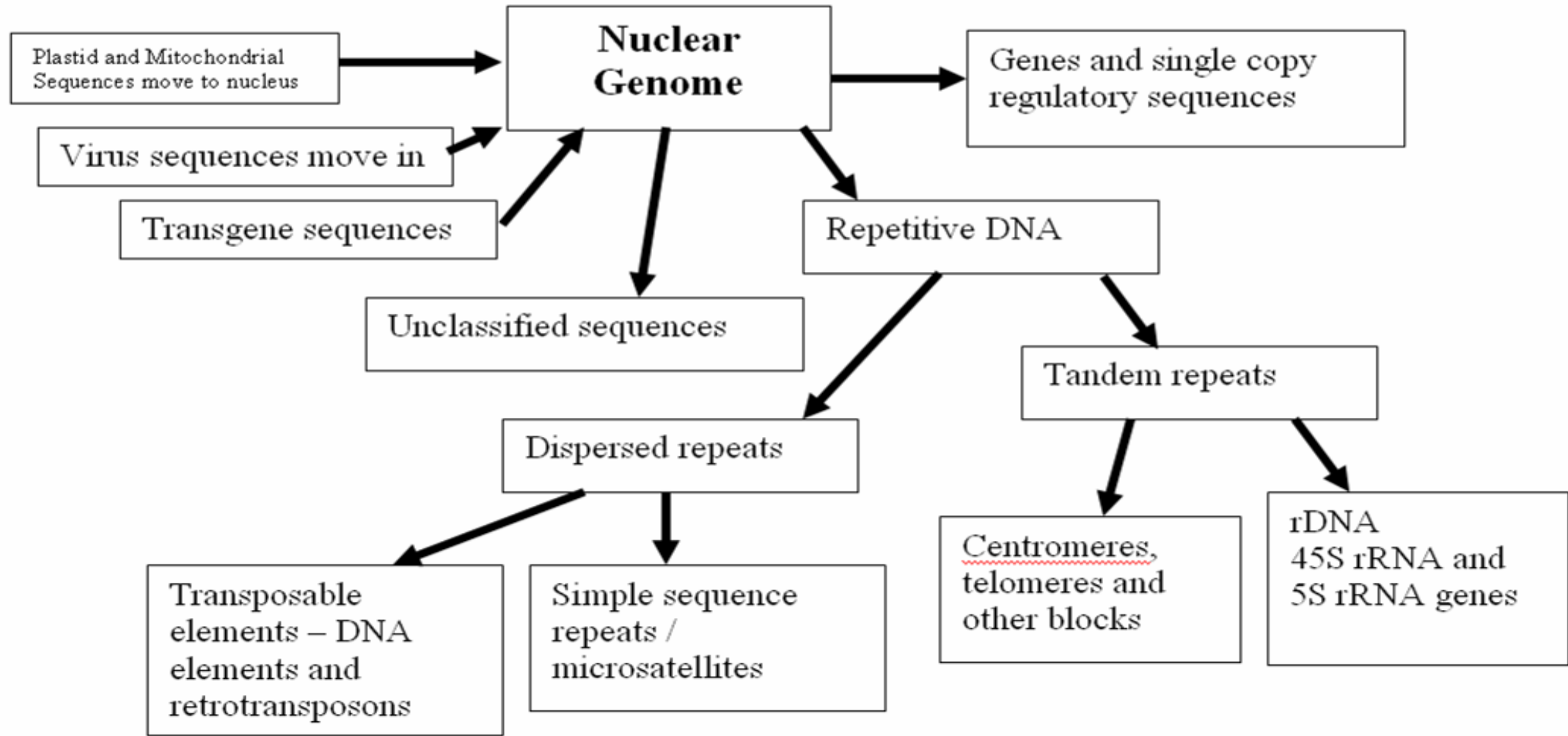
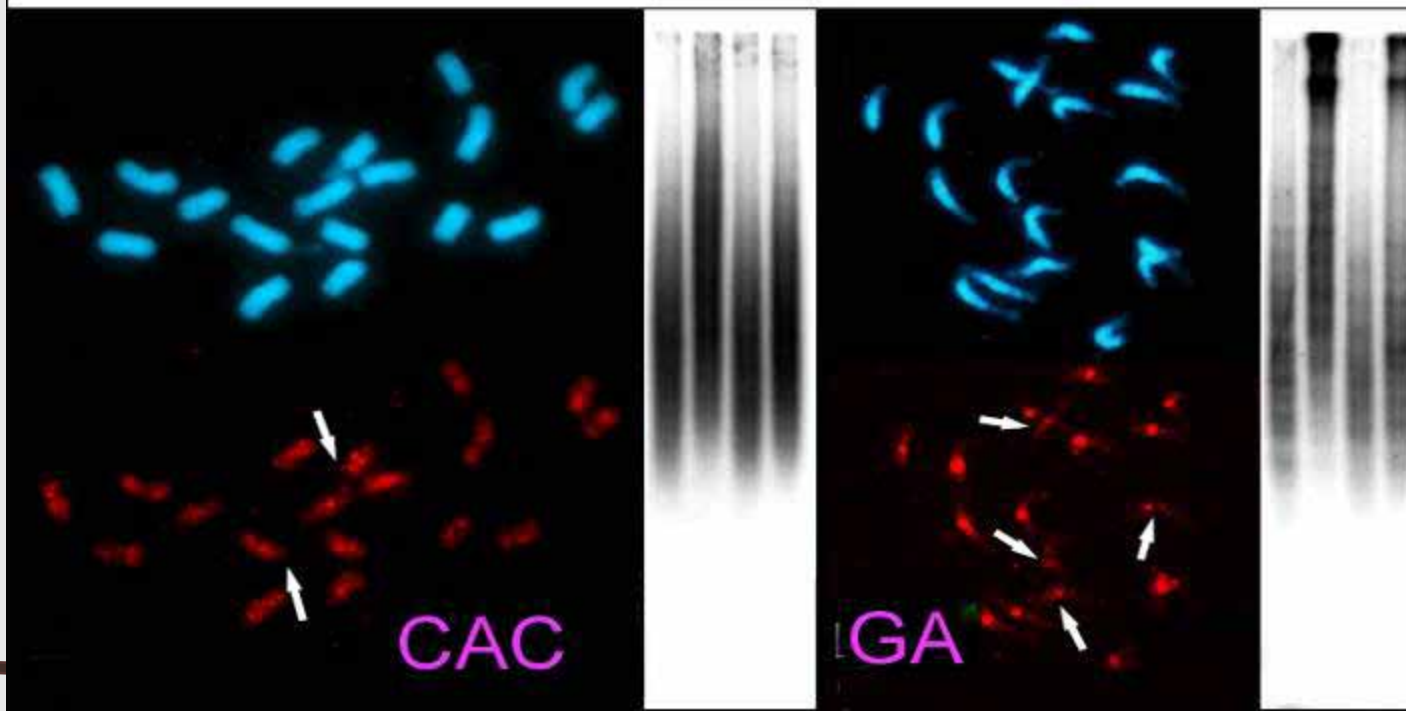
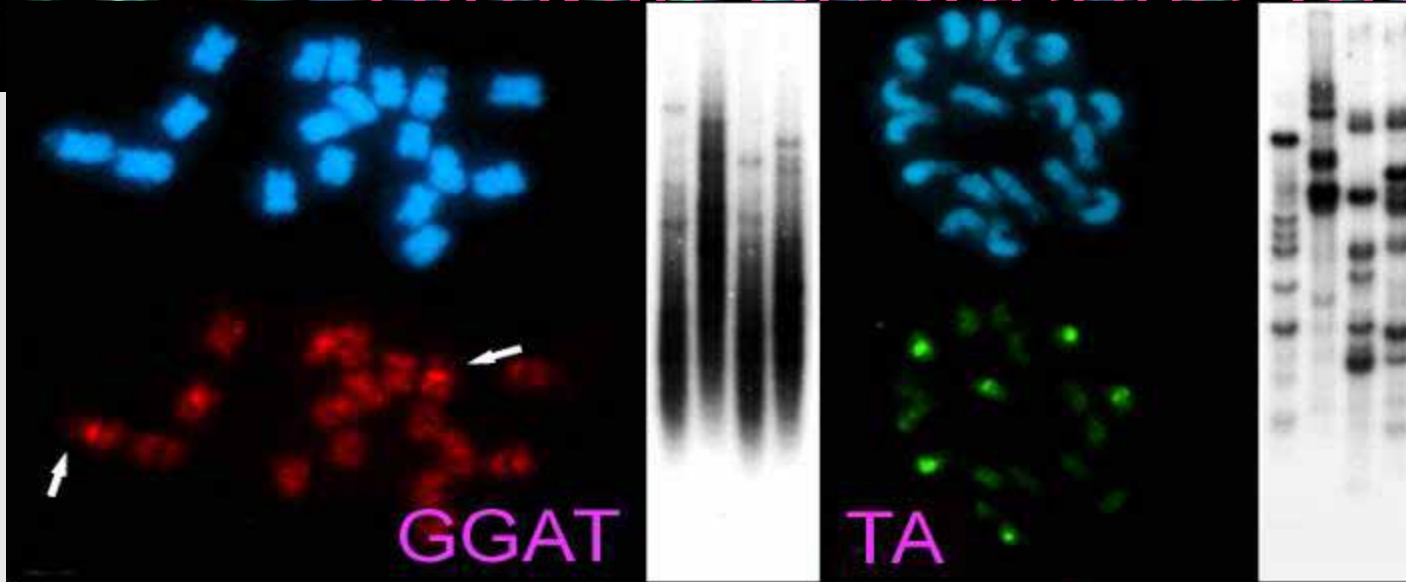


Figure 2. Components of the Nuclear Genome and their Relationships

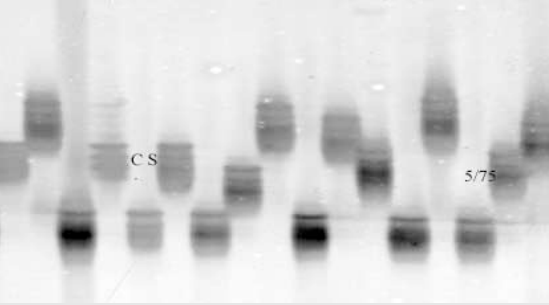


Simple Sequence Repeats



Sugar beet:
Characteristic
organization of
each motif

Schmidt, HH et al



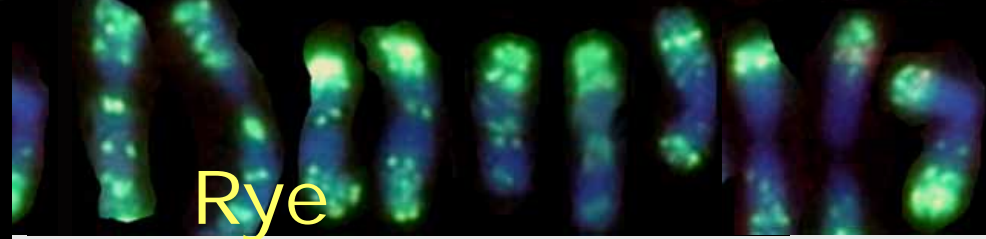
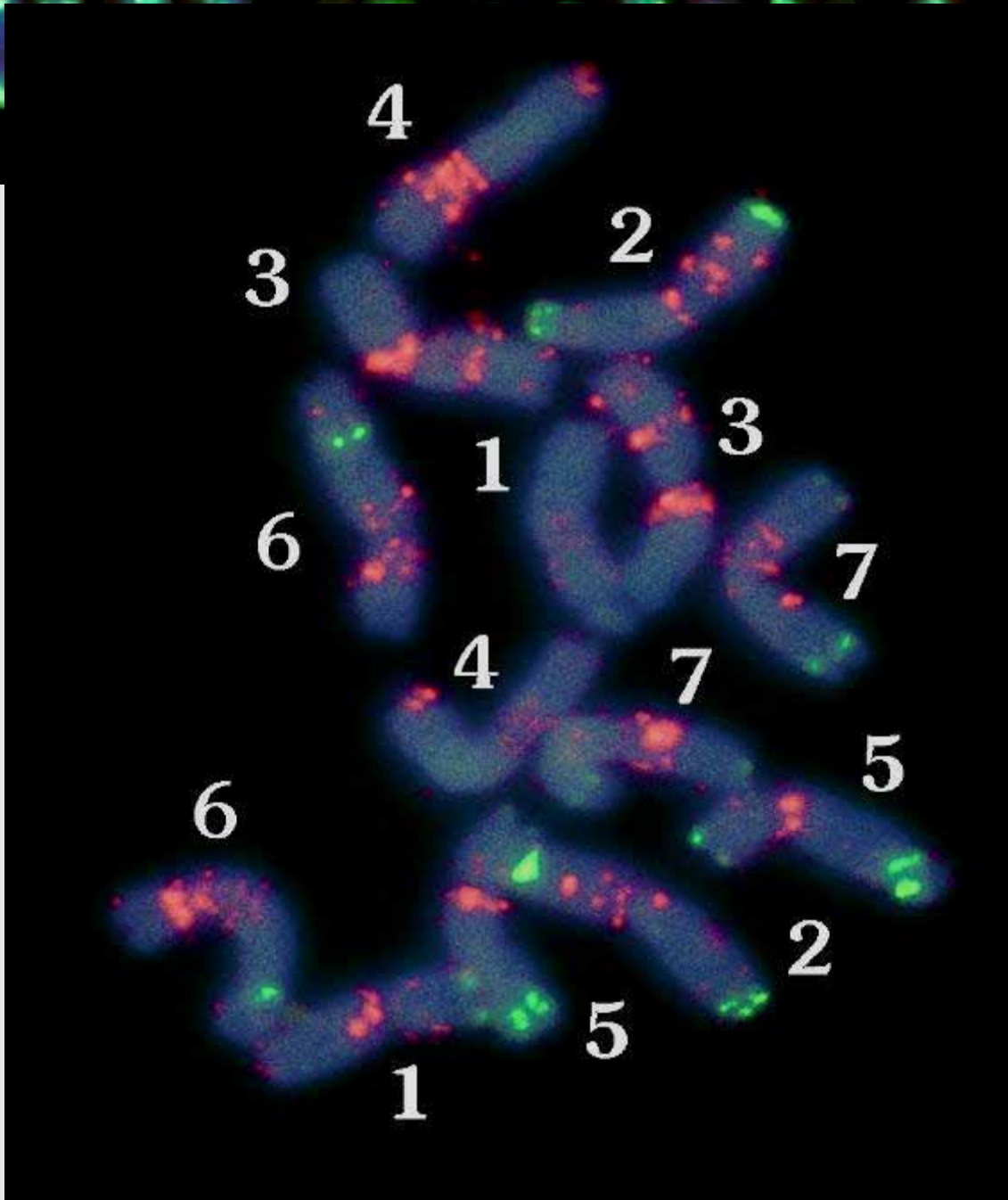
Microsatellites Simple Sequence Repeats (SSRs)

(GA)₇



(GA)₅

- Short (1-6bp), tandem repeats (10-50 copies)
- Mono- to tetra-nucleotides, e.g. (AT)_n
- Random distribution assumed
- Primers designed for conserved flanking regions
- Variation in repeat number → polymorphism



Rye

Secale cereale

$2n=14$

DAPI

FITC/Alexa 488

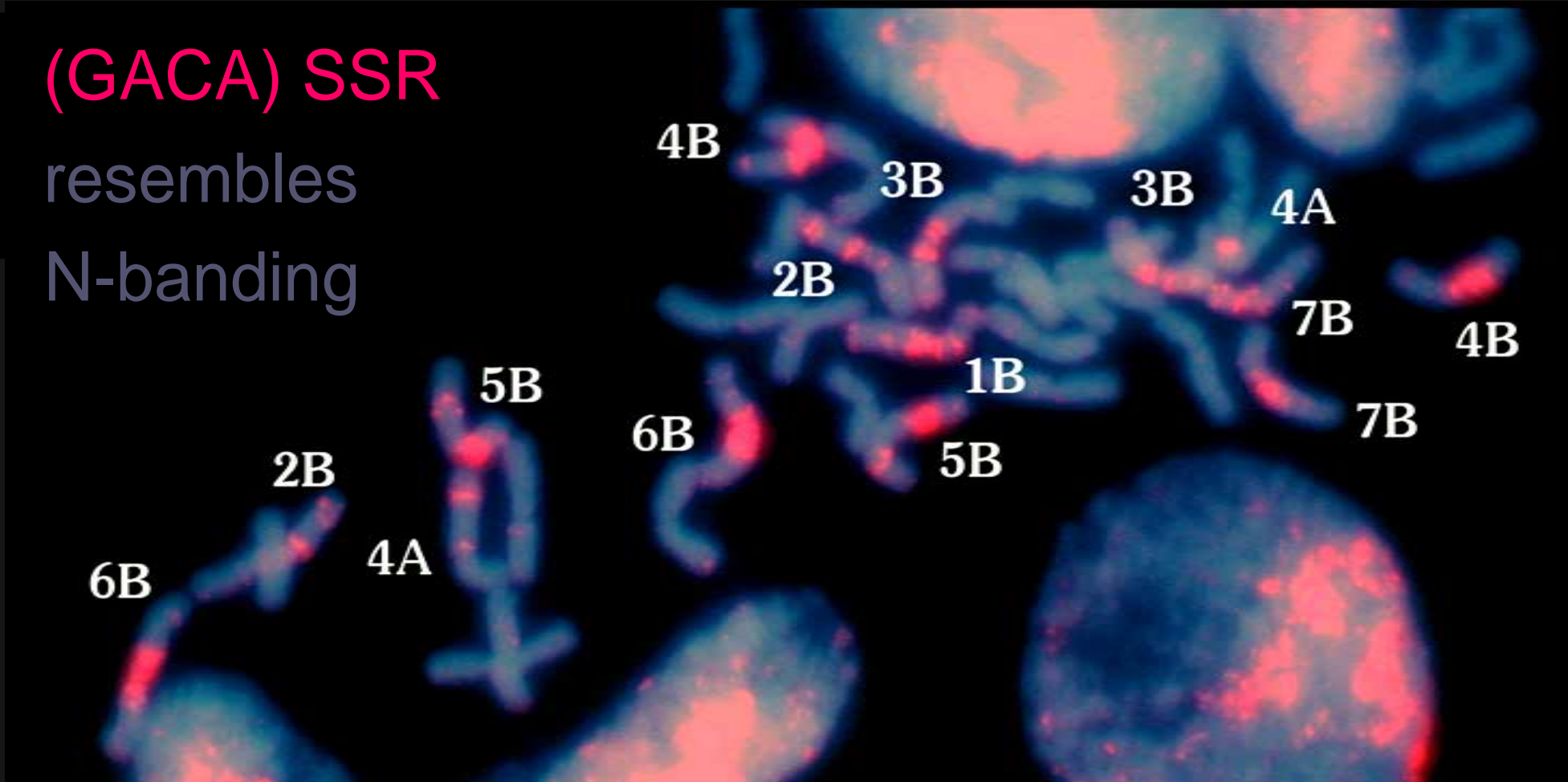
Cy3/Alexa 594

Wheat 'Chinese Spring'

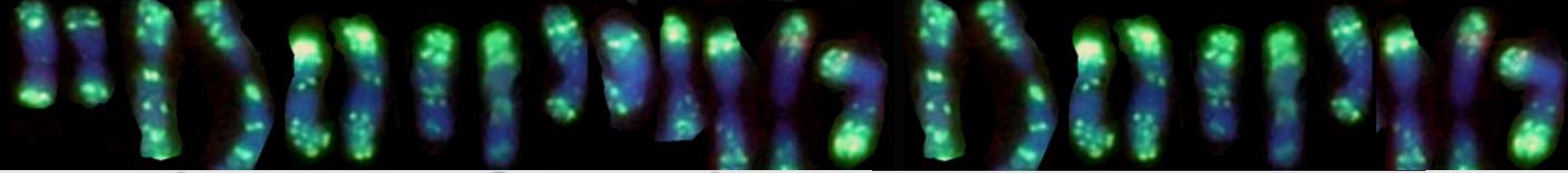
(GACA) SSR

resembles

N-banding



Cuadrado and Schwarzacher 1998

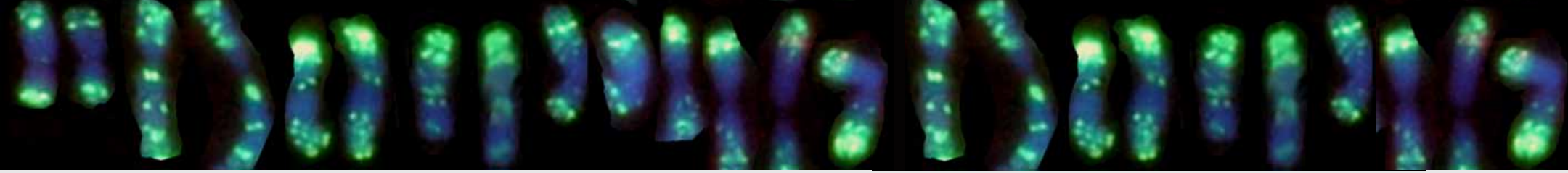


Clusters of Simple Sequence Repeats in rye and wheat

- In related chromosome position irrespective of motif used
- Wheat: resembles heterochromatic banding
- Rye: resembles wheat banding pattern, but not in major subtelomeric heterochromatic bands

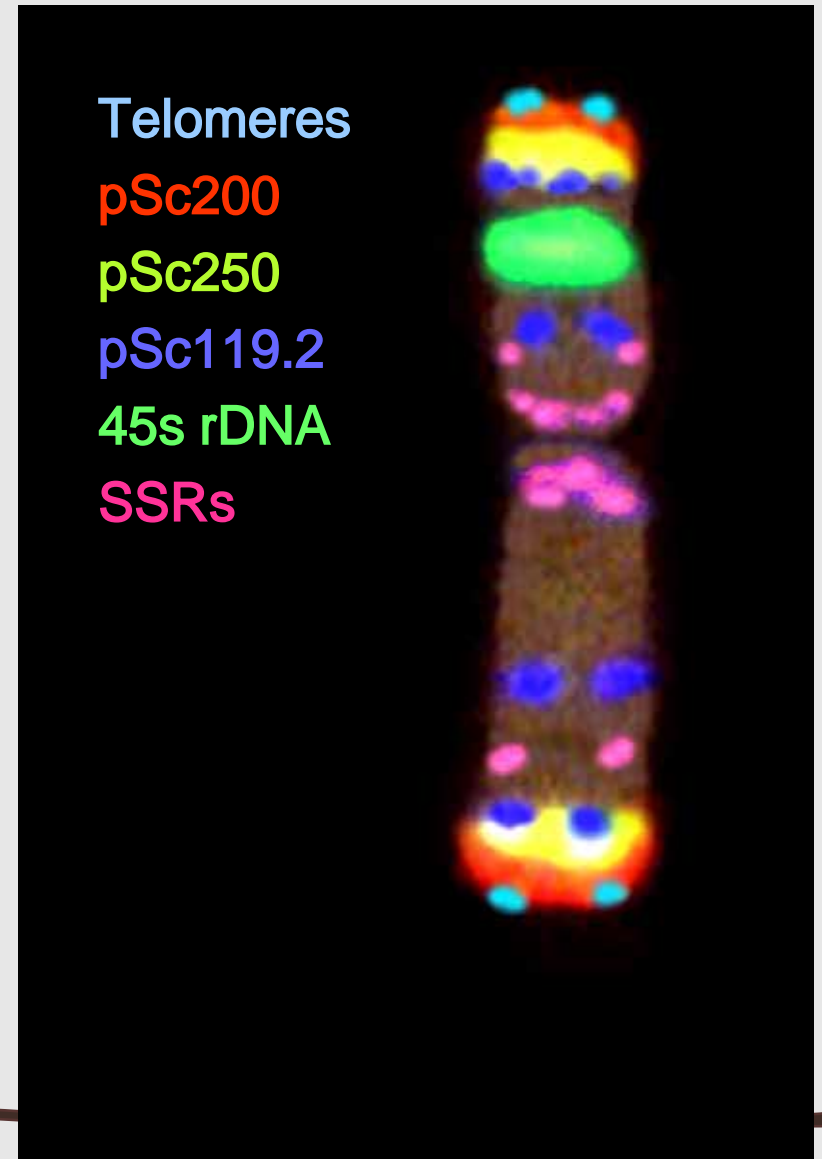
Ancient components of the Triticeae genome

Heterochromatin in wheat and rye not the same

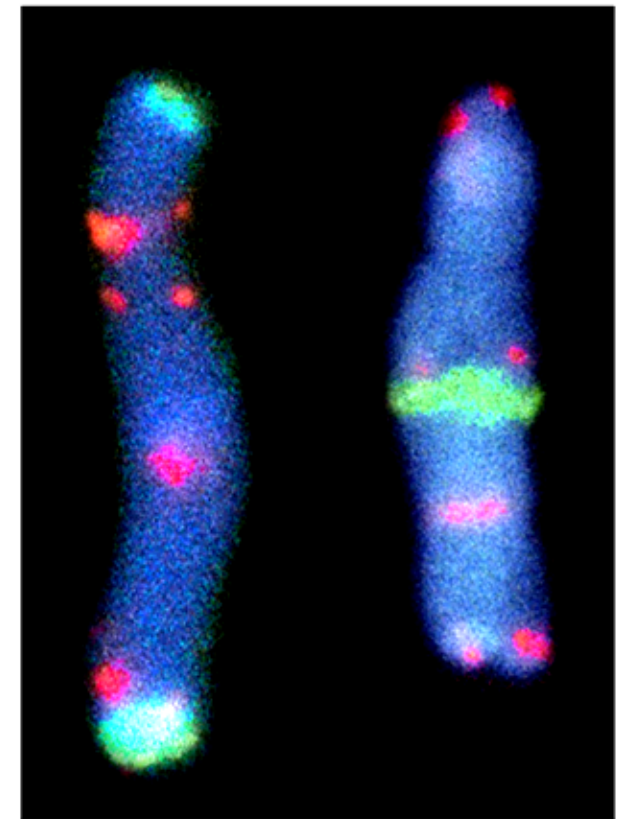
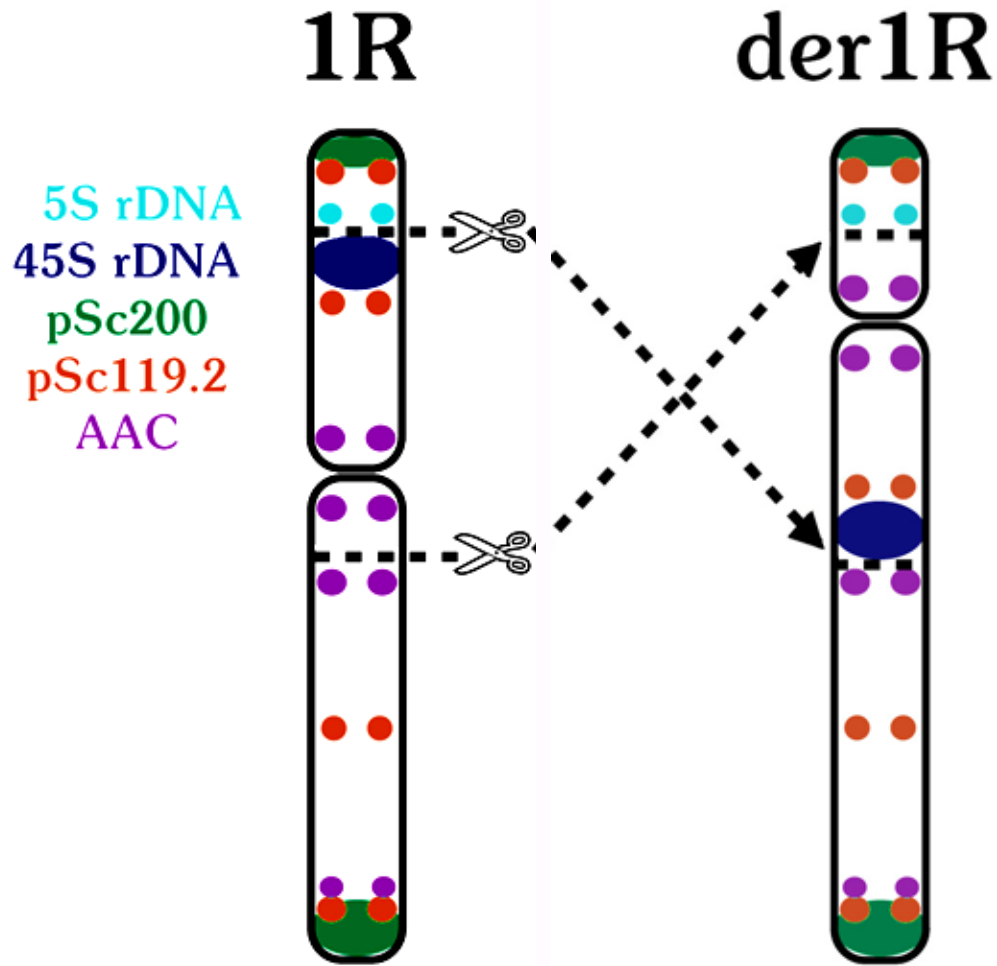


Chromosome model

1R of rye



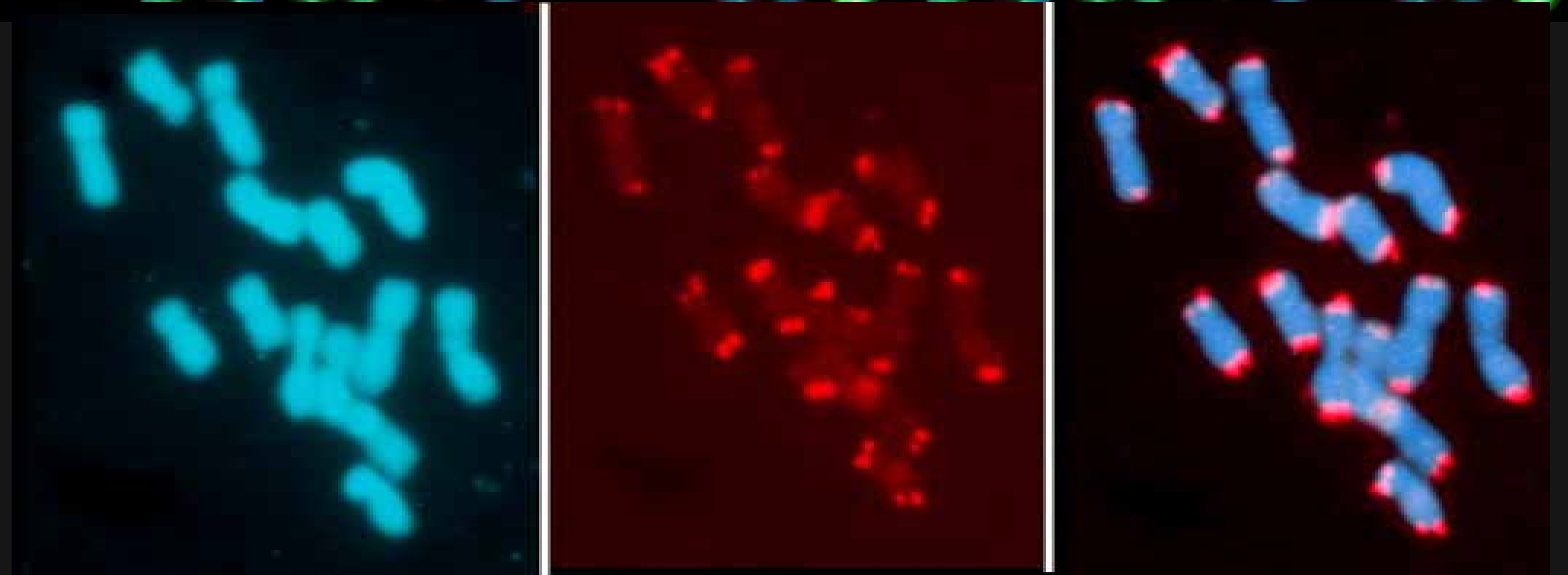
Derivative chromosome 1R of Lines 7-102 and 7-169



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



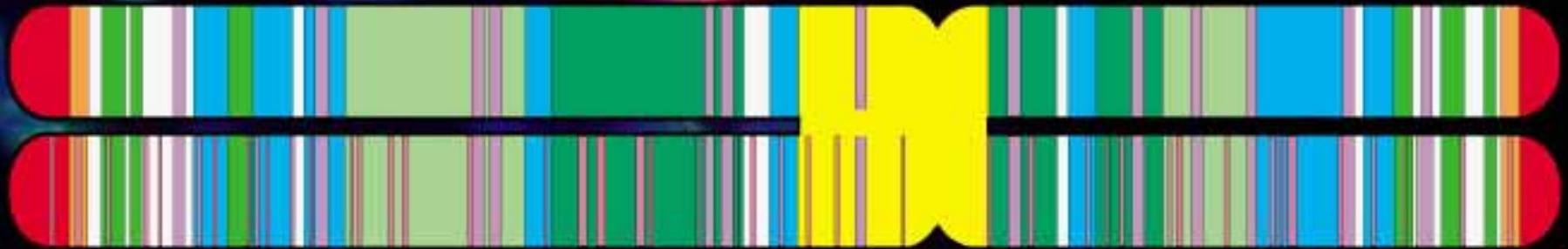
32 chromosomes DAPI;

TTTAGGG telomere;

45S rDNA (1 major pair + minor)

5S rDNA (1 major + minor)

The Linear Chromosome



Tandem repeats



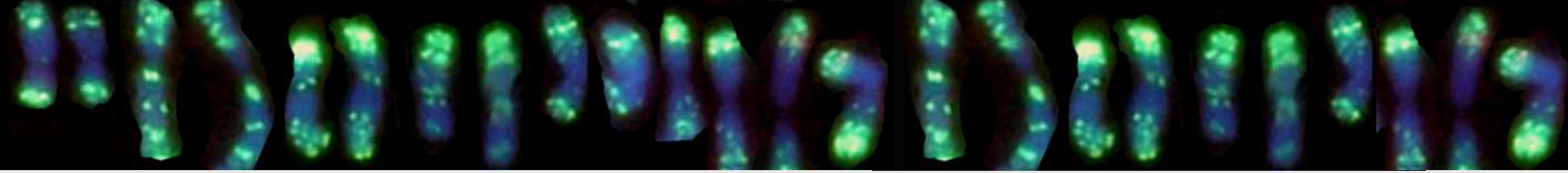
Terminal repeats



Retroelements
Simple sequence 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 Sequences – Fraction of the genome

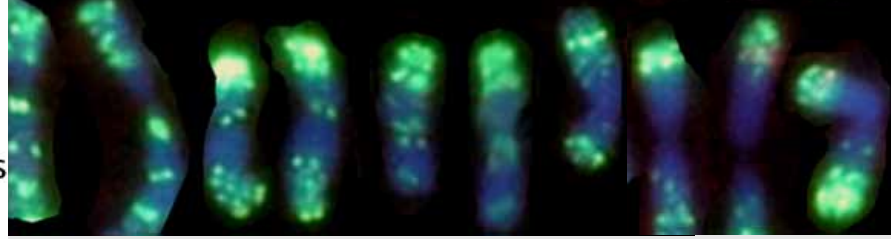
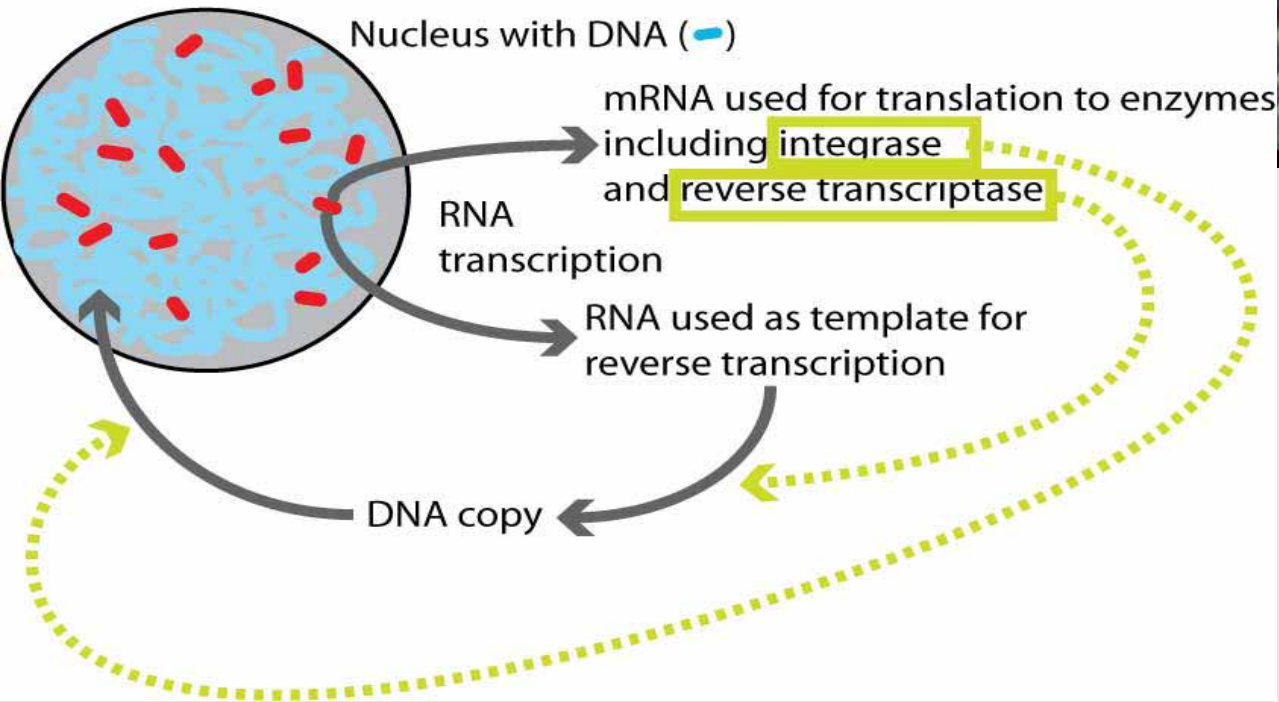
	Plant	Mammal
☞ Long terminal repeat		
● <i>copia</i>	25-50%	0%
● <i>gypsy</i>	5-25%	0%
☞ LINEs	10%	30%
● SINEs		
☞ Retroviral	<0.1%	8.3%



Retroelement Organization

- ☛ Amplify via RNA intermediate
- ☛ Full sequences and sequence remnants
- ☛ Wide genome dispersal
- ☛ Rapid evolution
- ☛ Activated under stress conditions

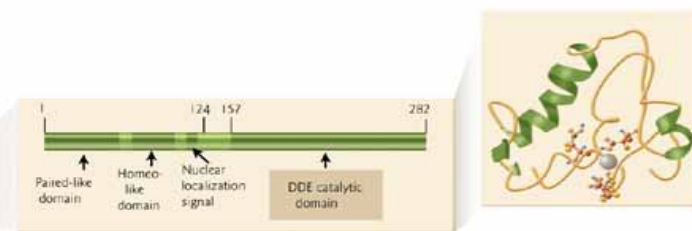
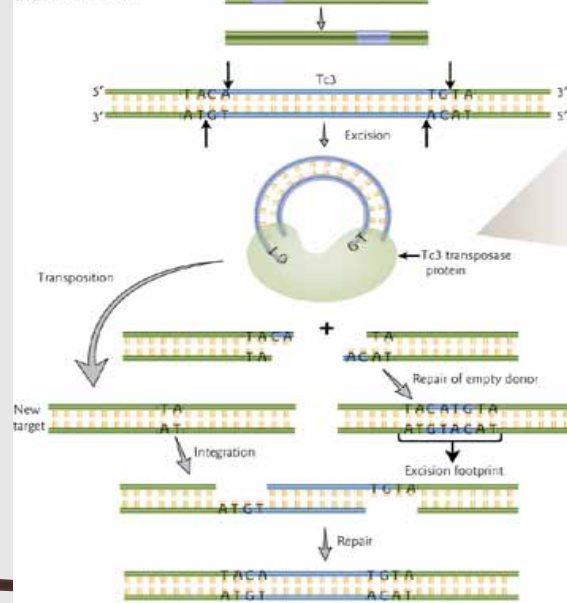
Retrotransposons (-): The transposition cycle



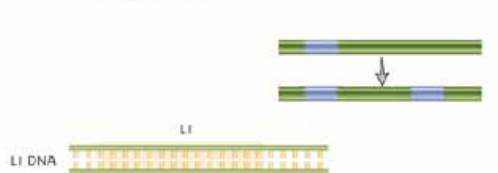
DNA transposons
Class II transposable elements
Cut-and-paste

Retrotransposons
Class I transposable elements
RNA intermediate

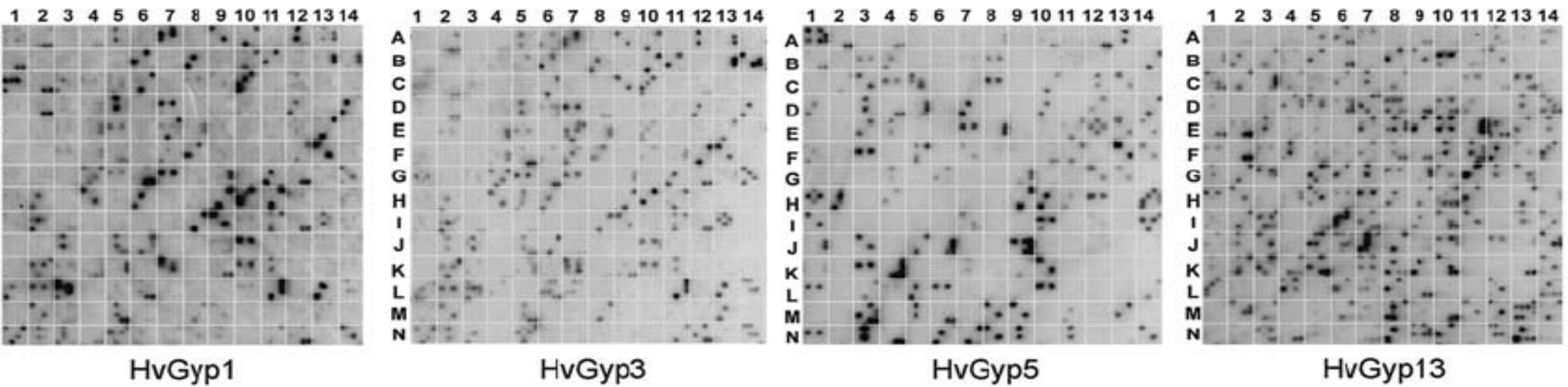
(A) Cut & Paste



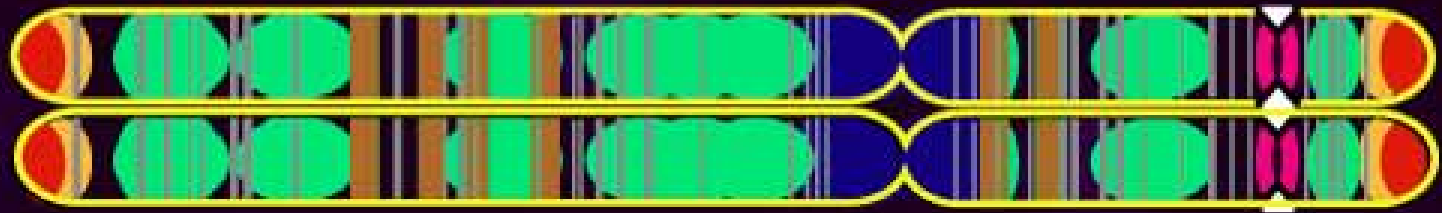
(B) Copy & Paste

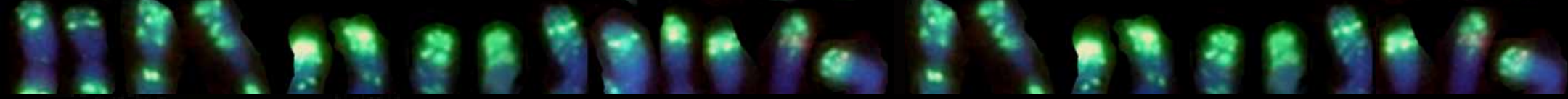


Retroelement abundance and diversity in barley

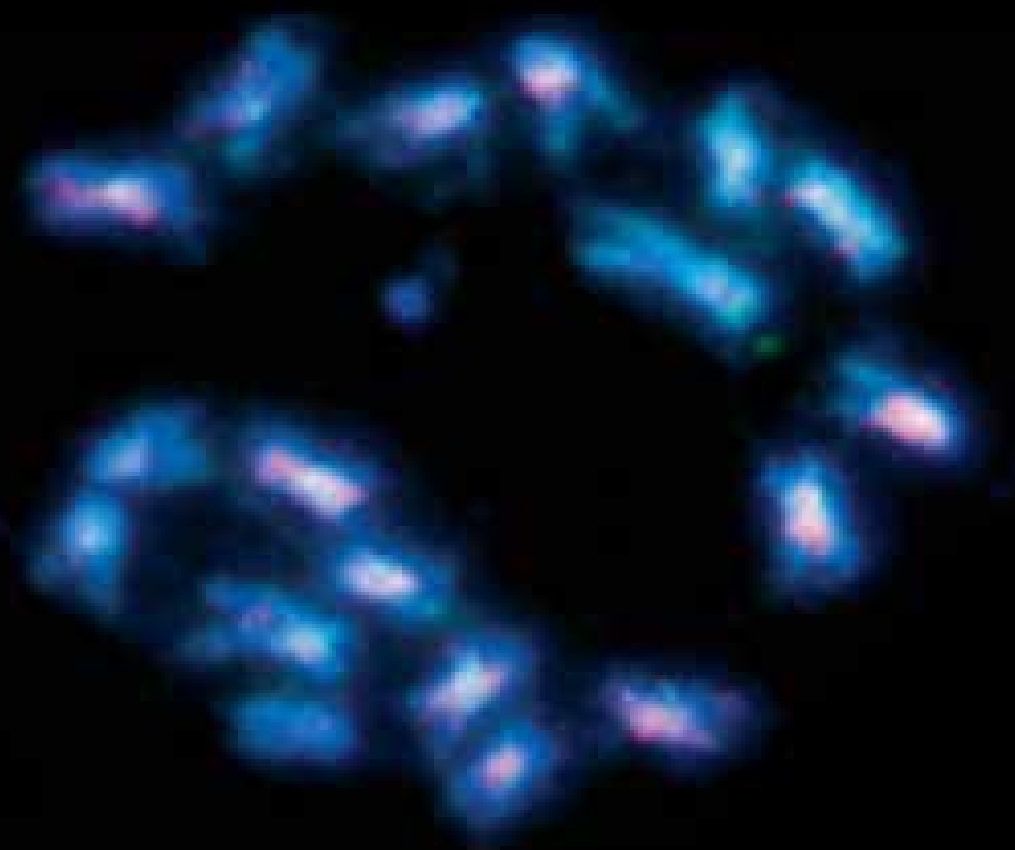
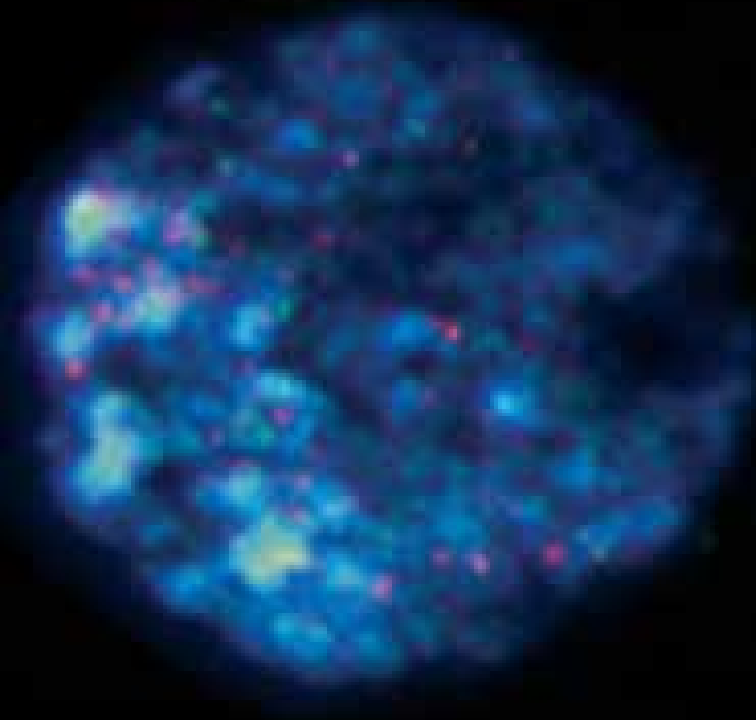


Gypsy elements are present in 25% of all BAC clones





PLANT
MOLECULAR
BIOLOGY



 International Society for
Plant Molecular Biology

 Springer

Retroelements in Brassica



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

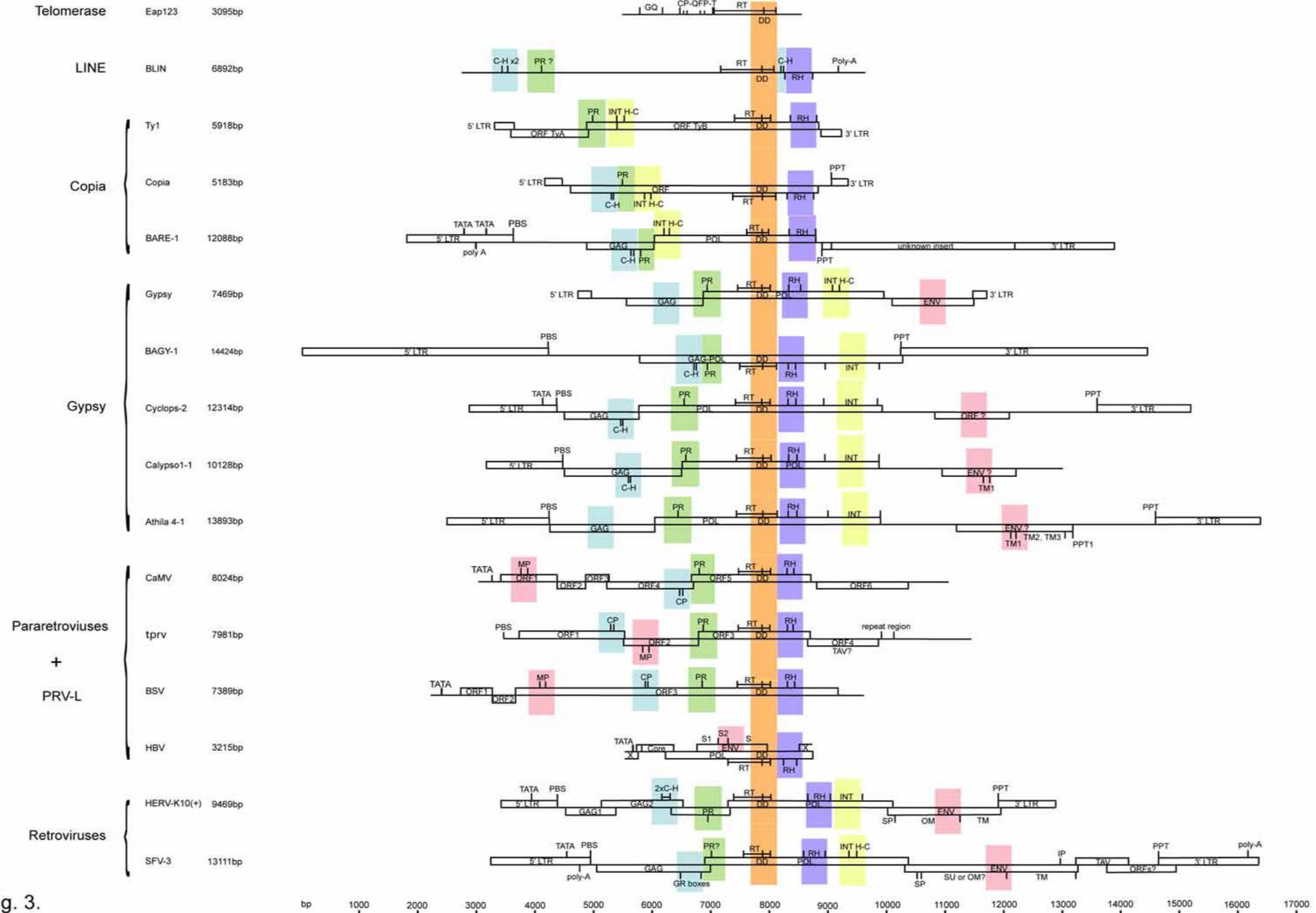
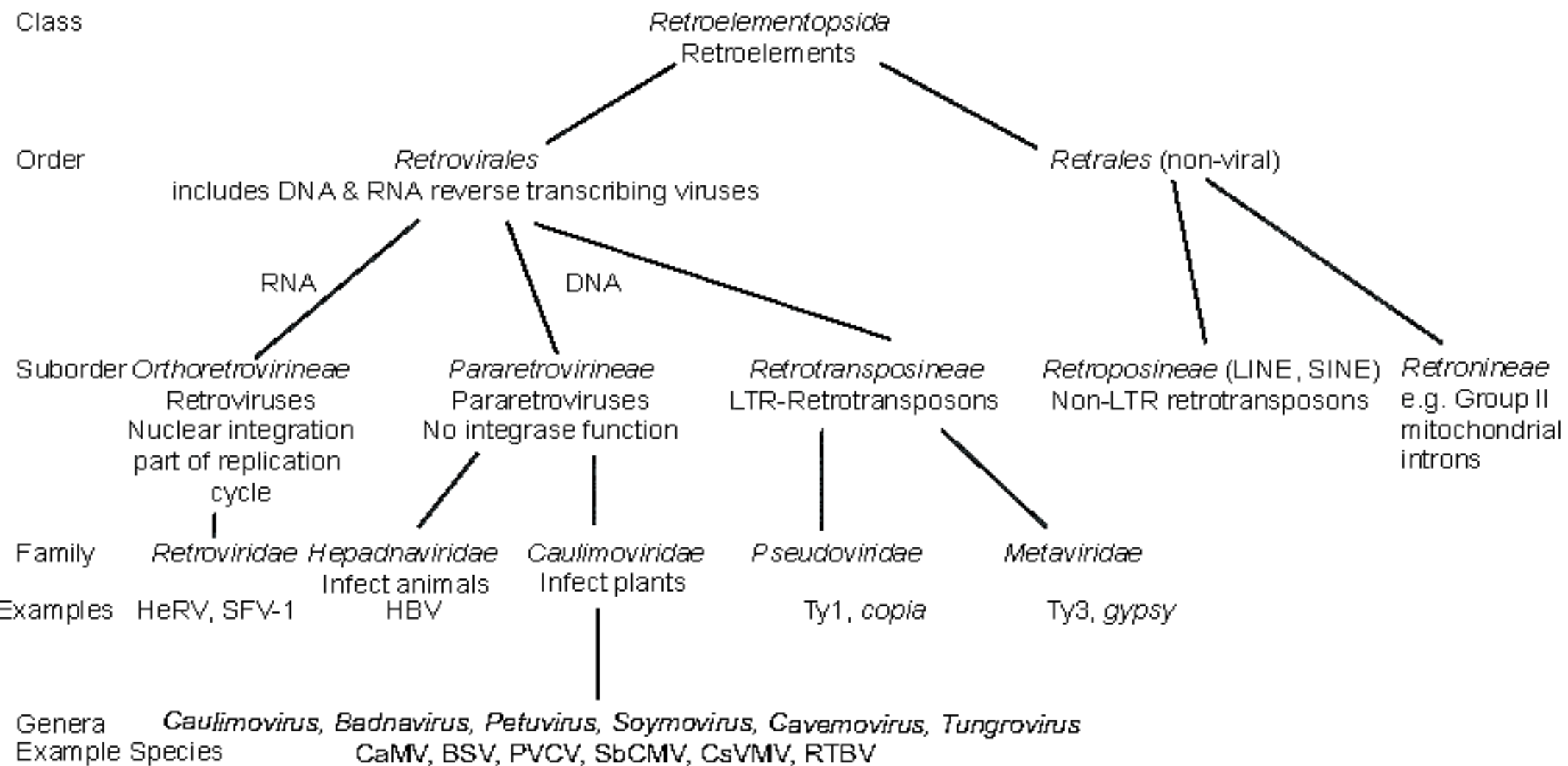
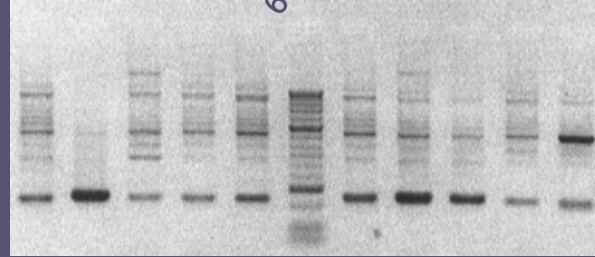
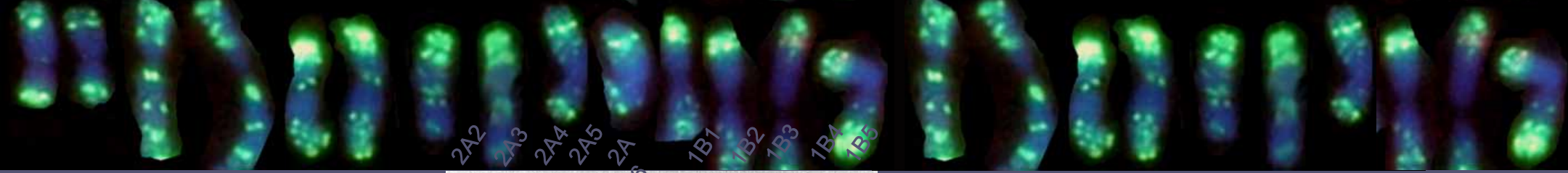


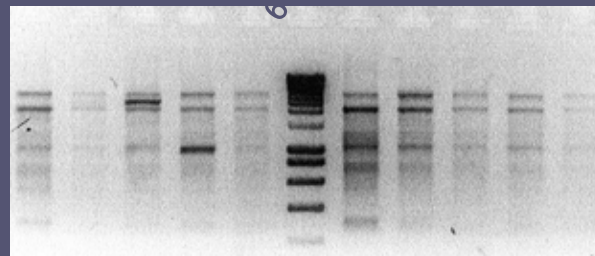
Fig. 3.



After Celia Hansen and J.S. Heslop-Harrison. 2004. www.molcyt.com After. *Advances in Botanical Research*



(a) Copia
Family: *Pseudoviridae*
(LTR-Retrotransposons)

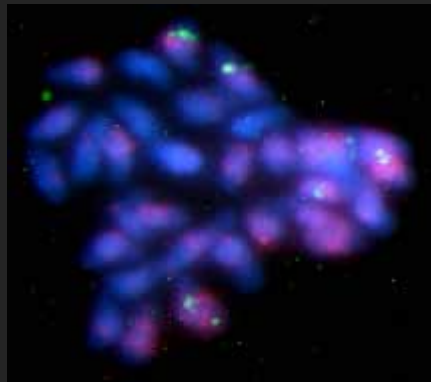
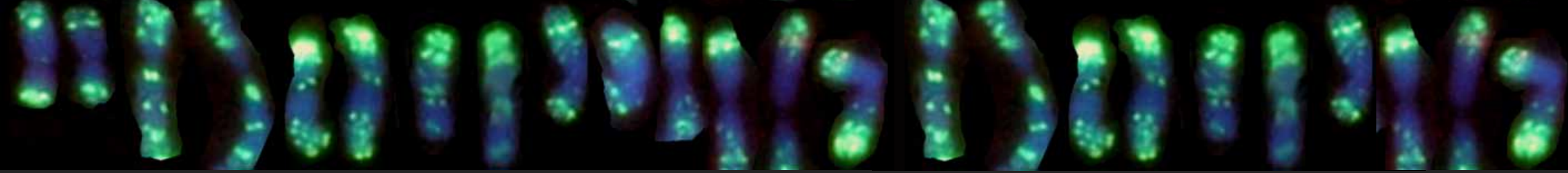


(b) Gypsy
Family: *Metaviridae*
(LTR-Retrotransposons)

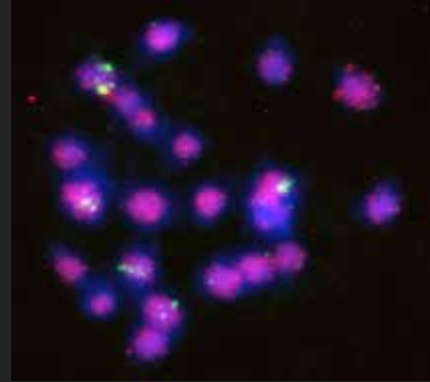


(c) LINE
(Non-LTR retrotransposons)

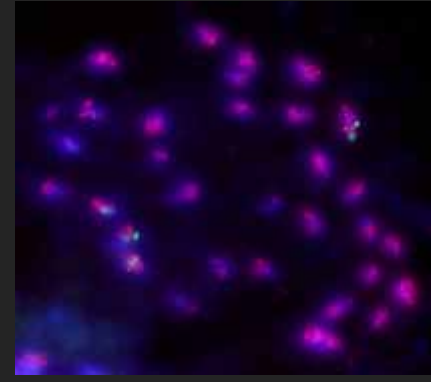
PCR products of (a) Copia primers (b) Gypsy primers (c) LINE primers obtained from 10 BAC samples of banana



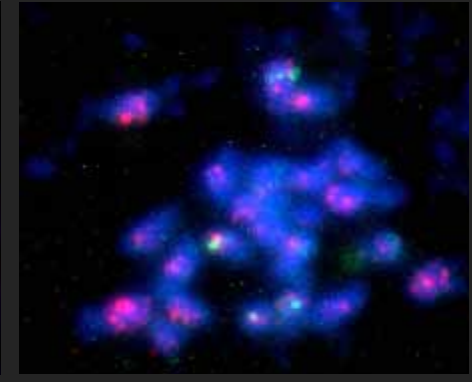
(a) Pisang Obino l'Ewai



(b) Pisang Mas

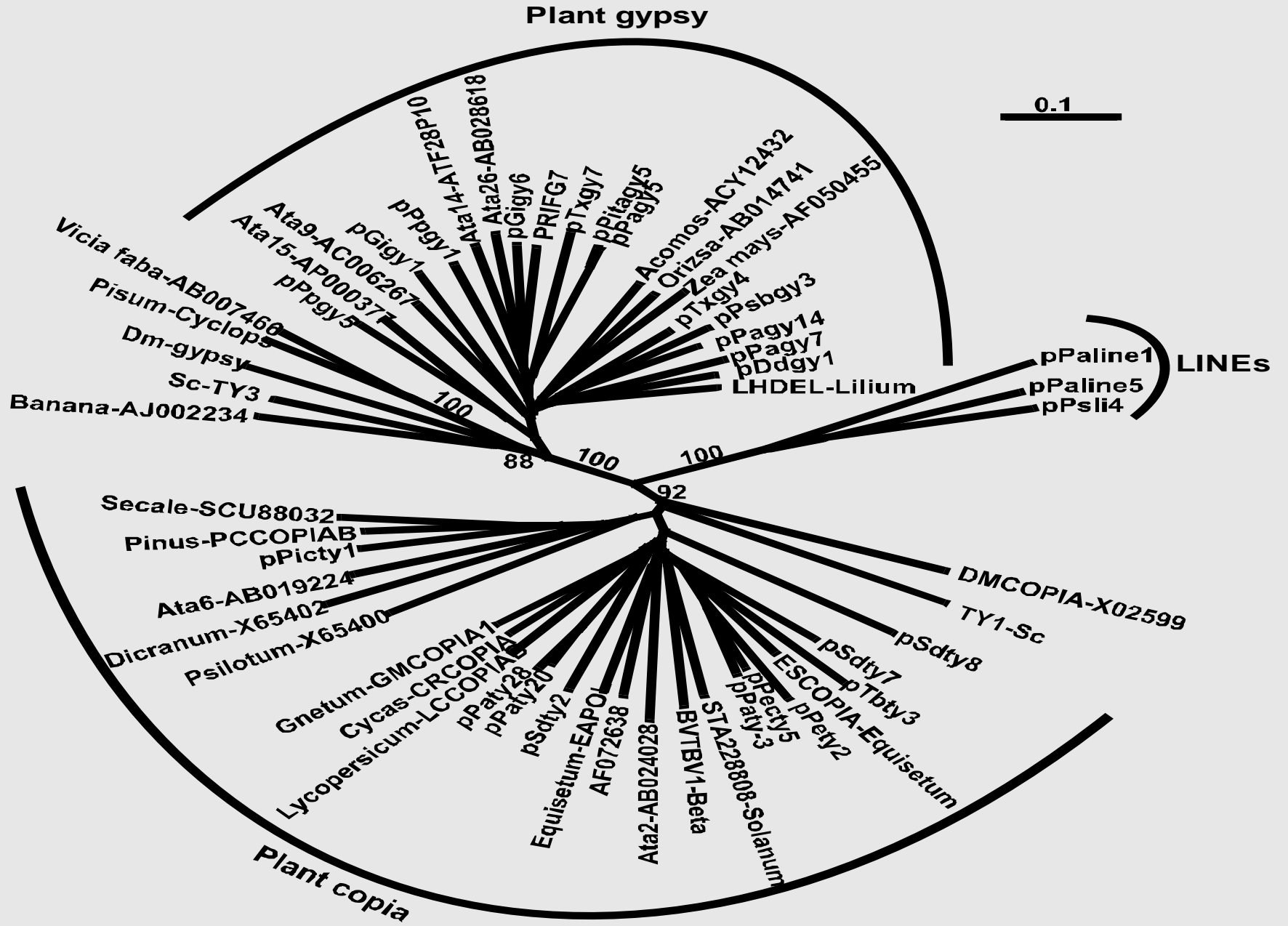


(c) Pisang Giant Cavendish



(d) Pisang Butuhan

Distribution of BAC2A3 and 5s rDNA on mitotic metaphase chromosome of Musa after FISH with labelled probes. BAC2A3 was labelled with biotin and pta794 was labelled with DIG.



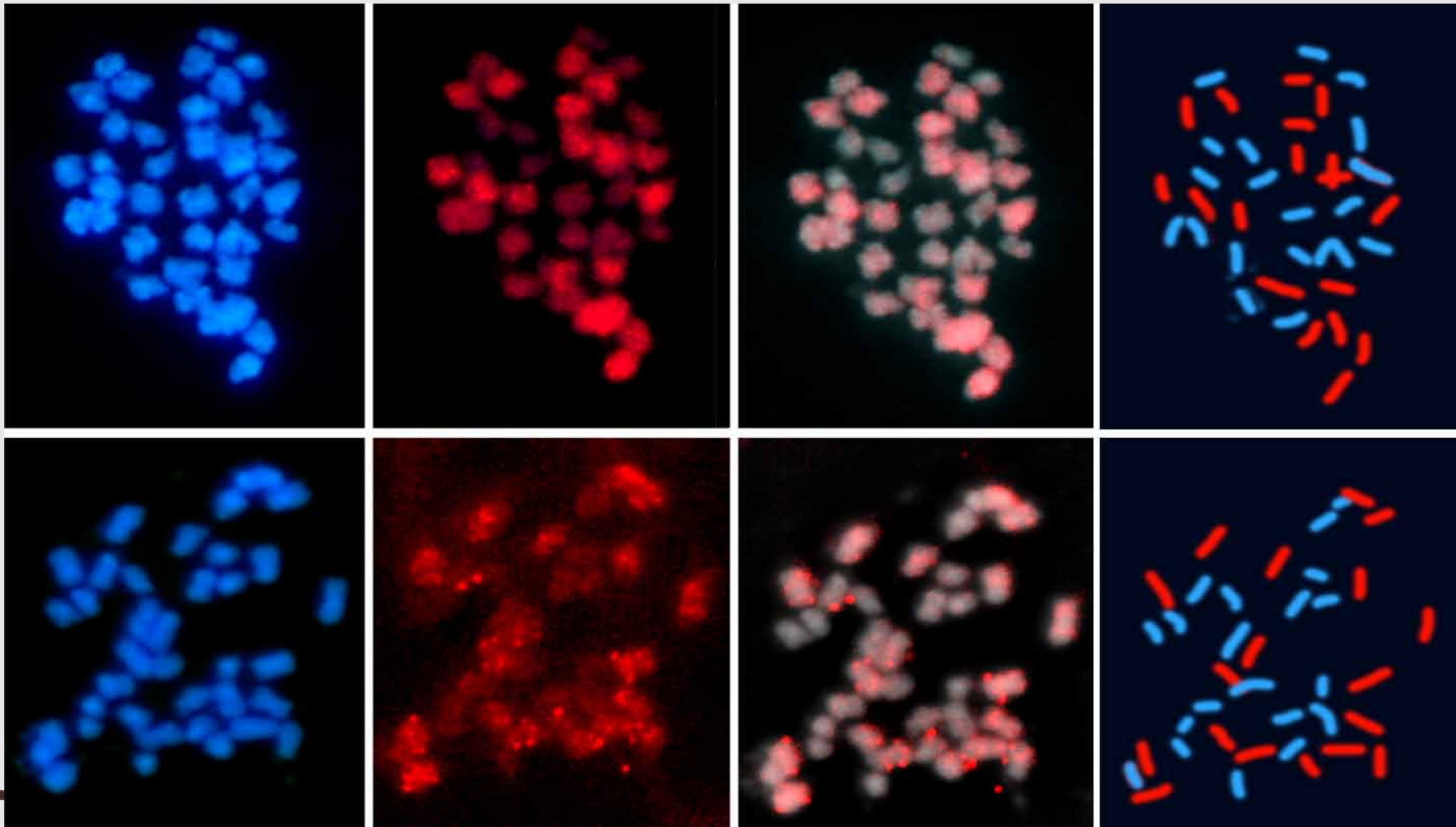
Genome Specificity of a CACTA (*En/Spm*) Transposon

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

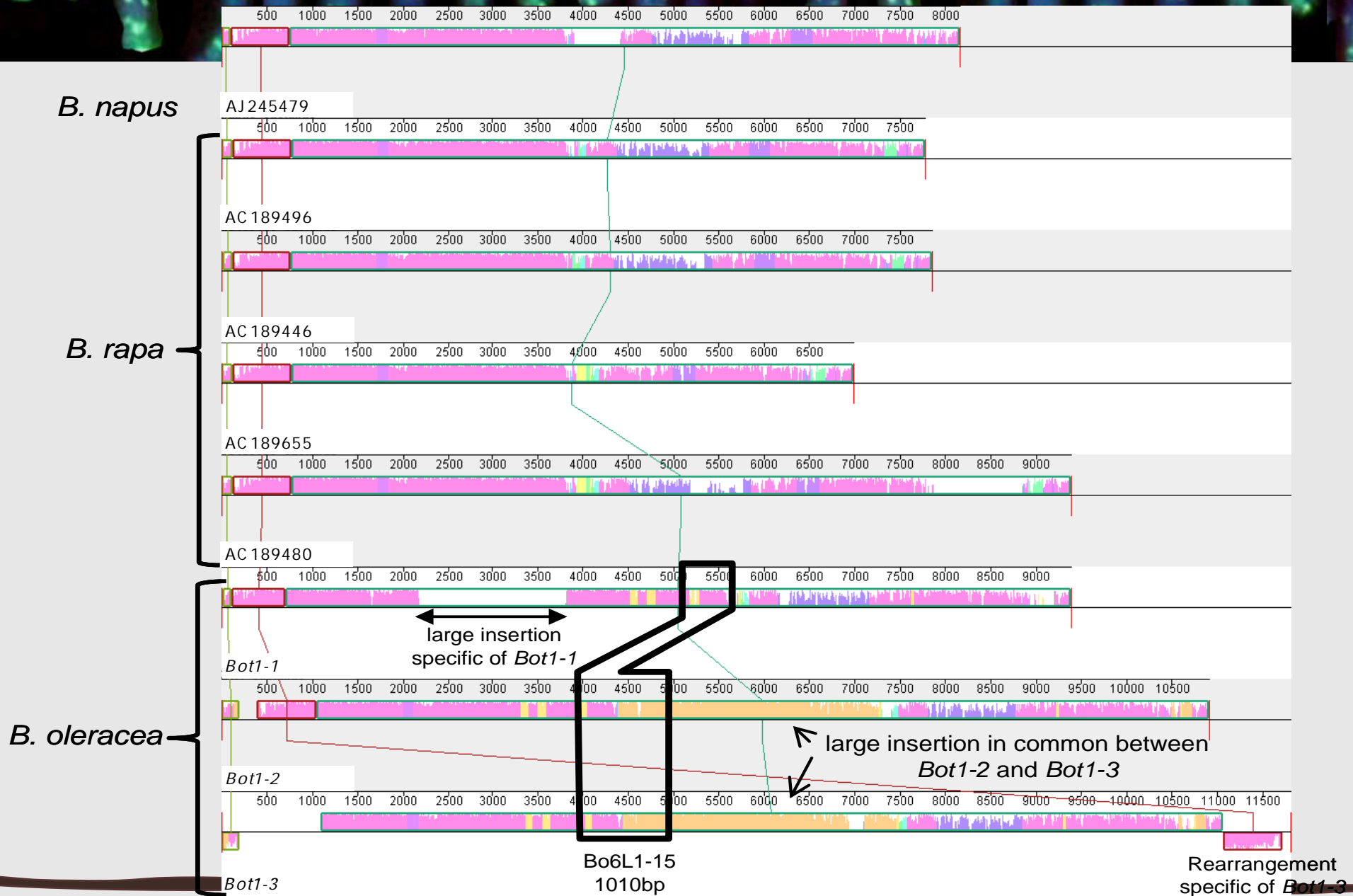
B. oleracea (CC, $2n=2x=18$)

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

Alix & HH 2008



Genome Specificity of a CACTA (En/Spm) Transposon

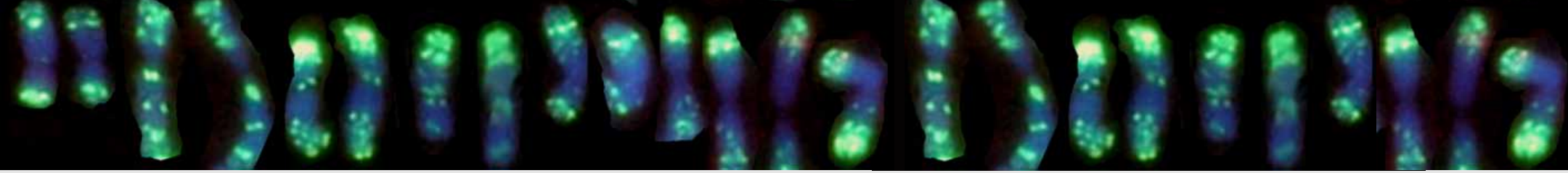




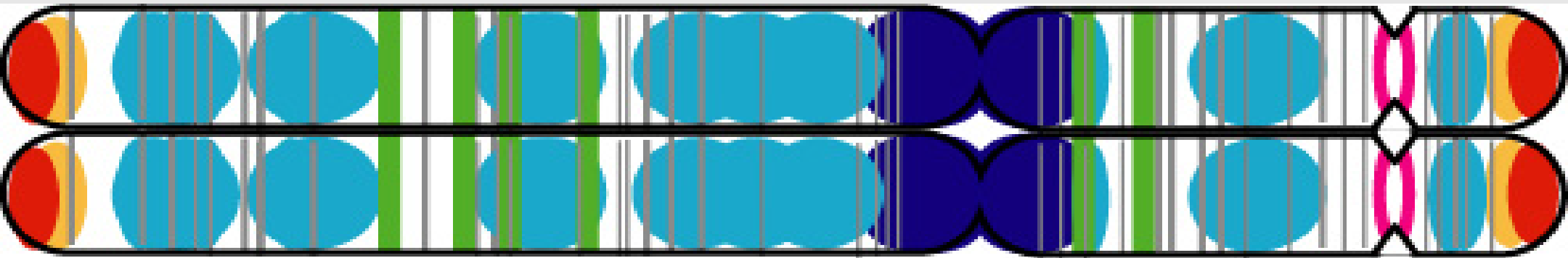
Genome Specificity of a CACTA (En/Spm) Transposon







- ☛ *Bot1* has encountered several rounds of amplification in the C (*B. oleracea*) genome only, playing a major role in the recent *B. rapa* and *B. oleracea* genome divergence
- ☛ *Bot1* carries a host S-locus associated *SLL3* gene copy; is the transposon associated with *SLL3* proliferation?

à Transposons are a driver of genome and genome evolution



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

Crocus species and hybrids



C. flavus

$2n=8$



C. 'Stellaris'

$2n=2x=10$



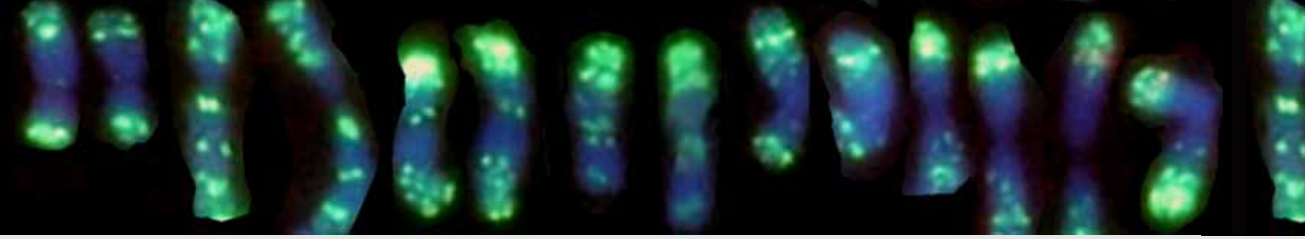
C. angustifolius

$2n=12$

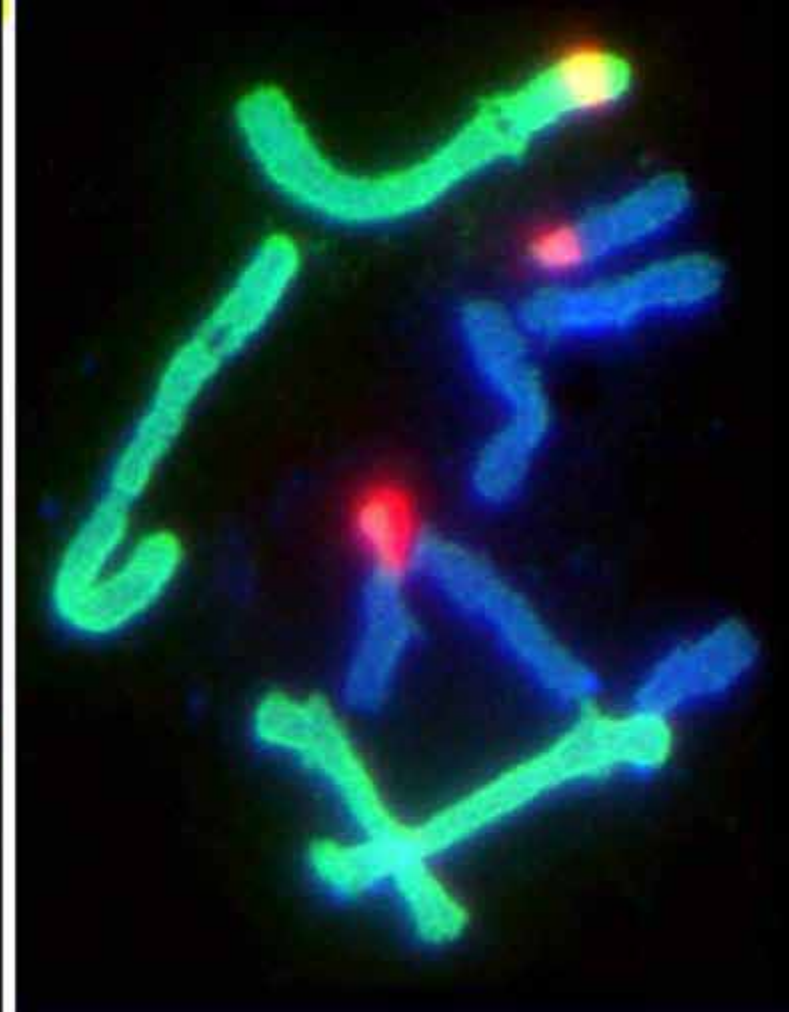
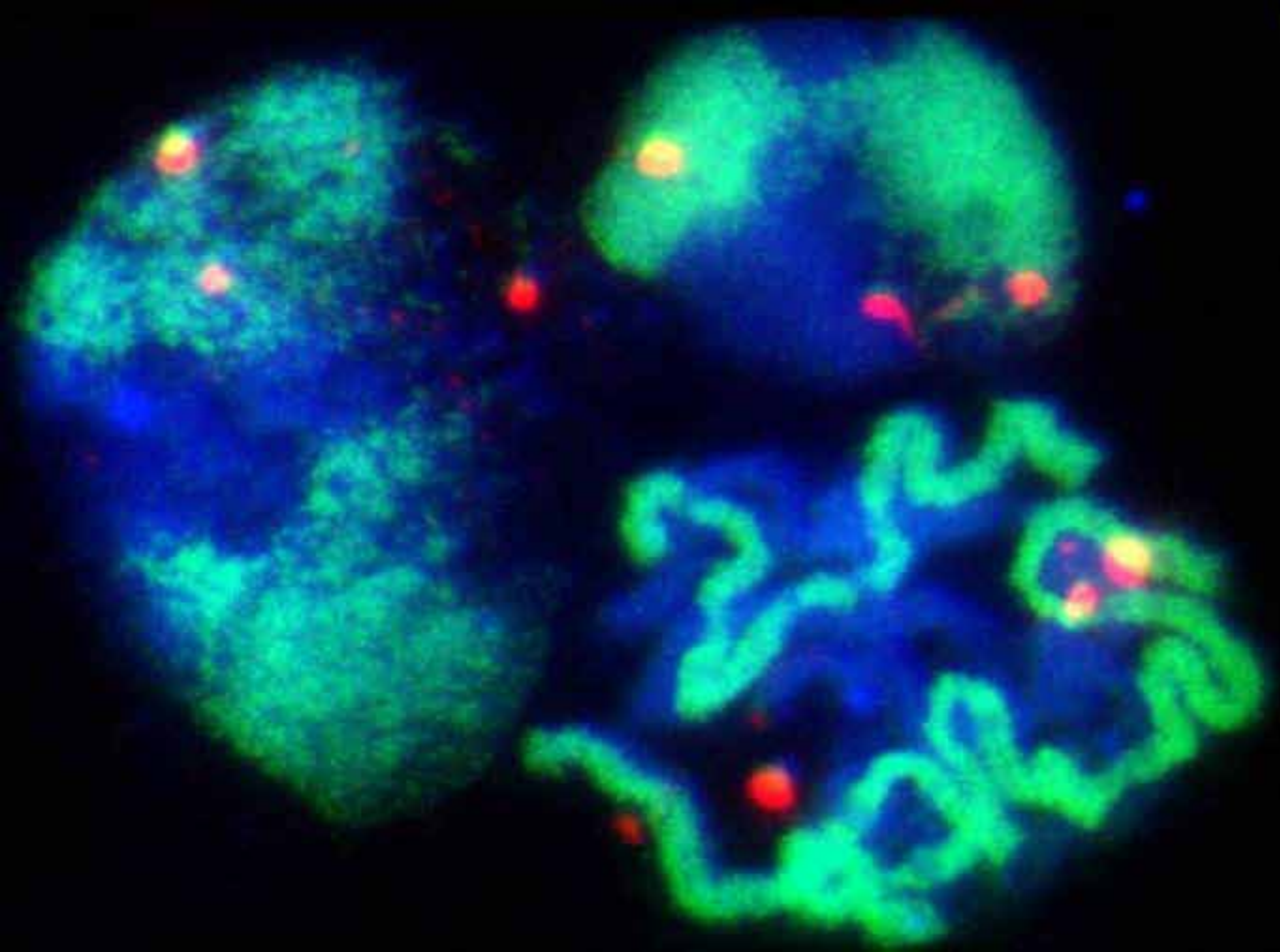


C. 'Golden Yellow'

$2n=3x=14$



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



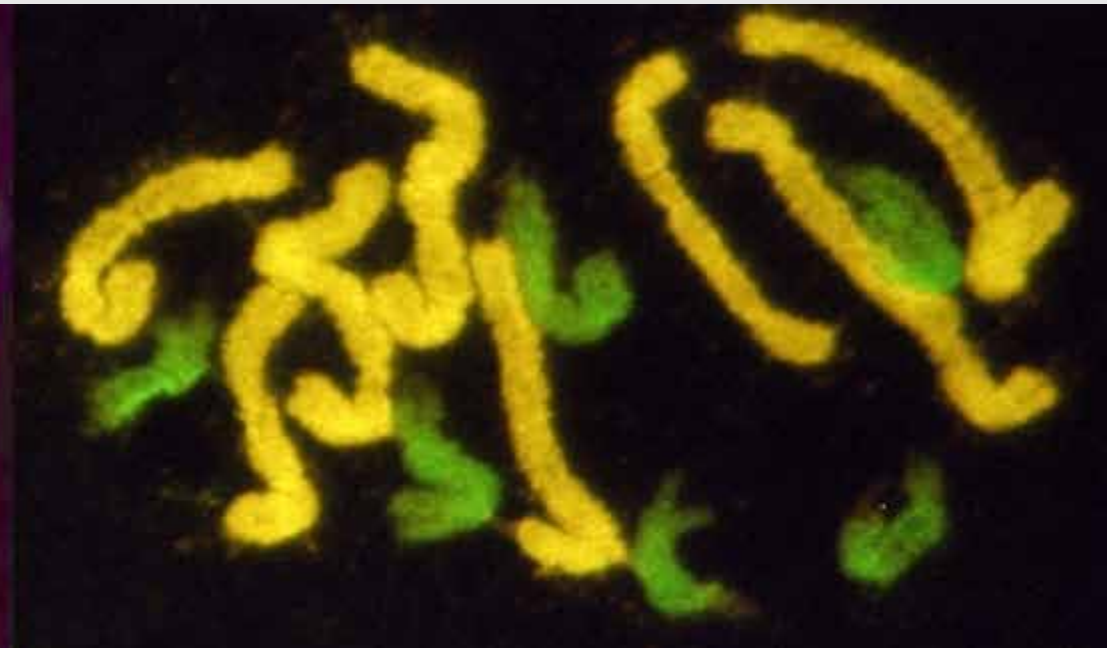
- ☛ The parents look similar, sharing many genes
- ☛ Total genomic DNA as a probe labels the parental genomes differentially



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

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

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



Orgaard, Jacobsen & HH





Polyploidy

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

☞ diploid $2n=2x$

☞ triploid $2n=3x$

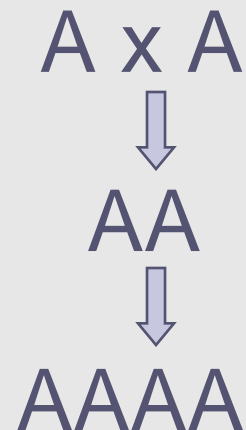
☞ hexaploid $2n=6x$

☞ tetraploid $2n=4x$

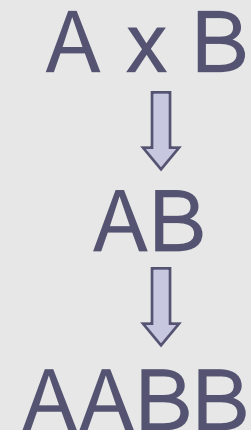
☞ octoploid $2n=8x$

- Two types of polyploidy

Autopolyploidy



Allopolyploidy



Wheat evolution and hybrids

Triticum uratu

$2n=2x=14$

AA

Aegilops speltoides
relative

$2n=2x=14$

BB

Triticum dicoccoides

$2n=4x=28$

AABB

Einkorn

Triticum monococcum

$2n=2x=14$

AA

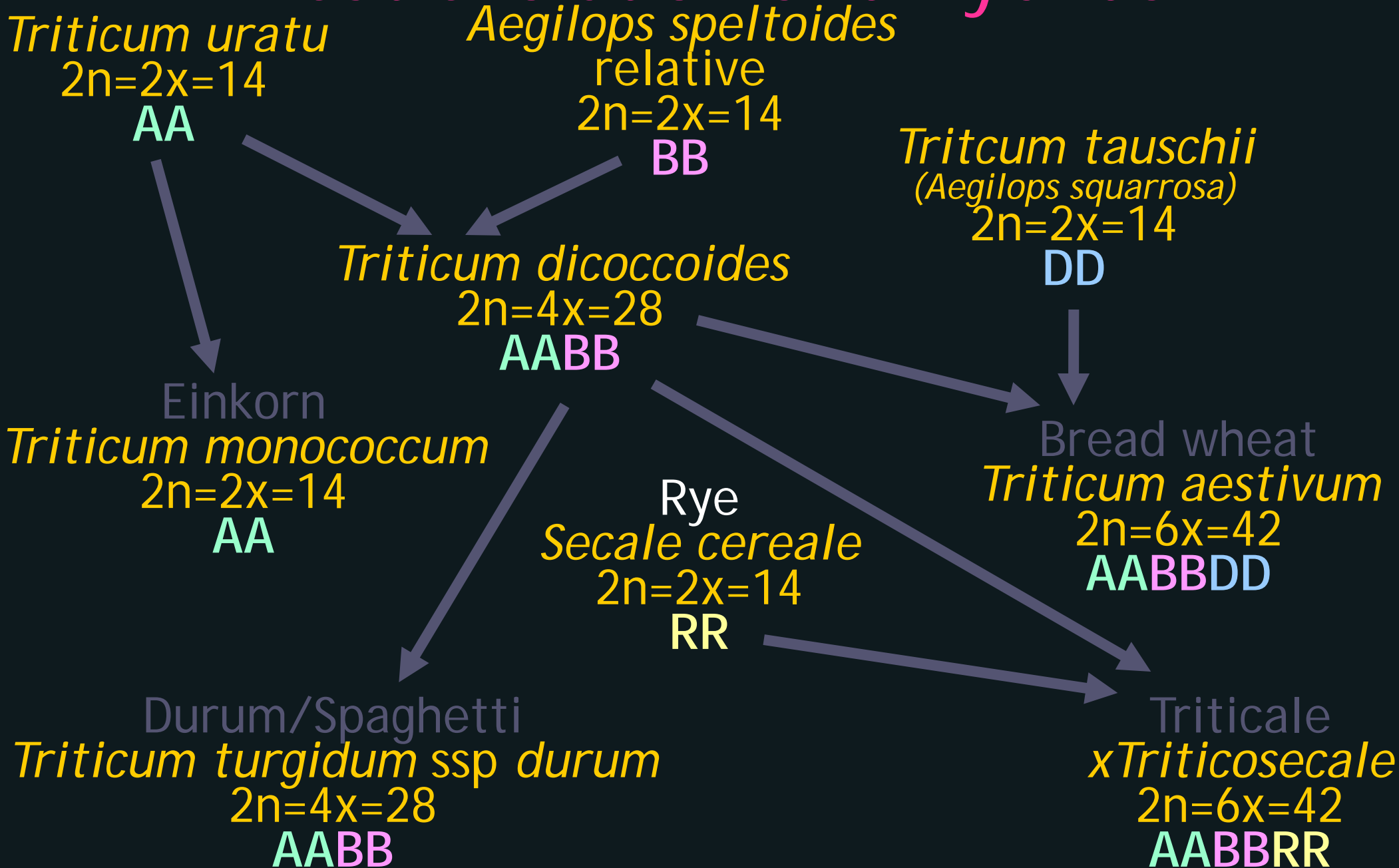
Durum/Spaghetti

Triticum turgidum ssp durum

$2n=4x=28$

AABB

Wheat evolution and hybrids



Aneuploidy and chromosome rearrangements

☞ Additions

- Monosomic addition (not stable)

- Plant: $2n = 42 + 1$
- Gametes: $n = 21$ or $21 + 1$
- Zygote: $2n = 42, 42 + 1, 42 + 2$

- Disomic addition (more stable)

- Plant: $2n = 42 + 2$
- Gametes: $n = 21 + 1$

☞ Substitutions (exchange of chromosomes)

☞ Deletions (chromosome is missing - nullisomic)

☞ Additions (extra chromosome – trisomic, tetrasomic)

☞ Nulli/Tetra lines

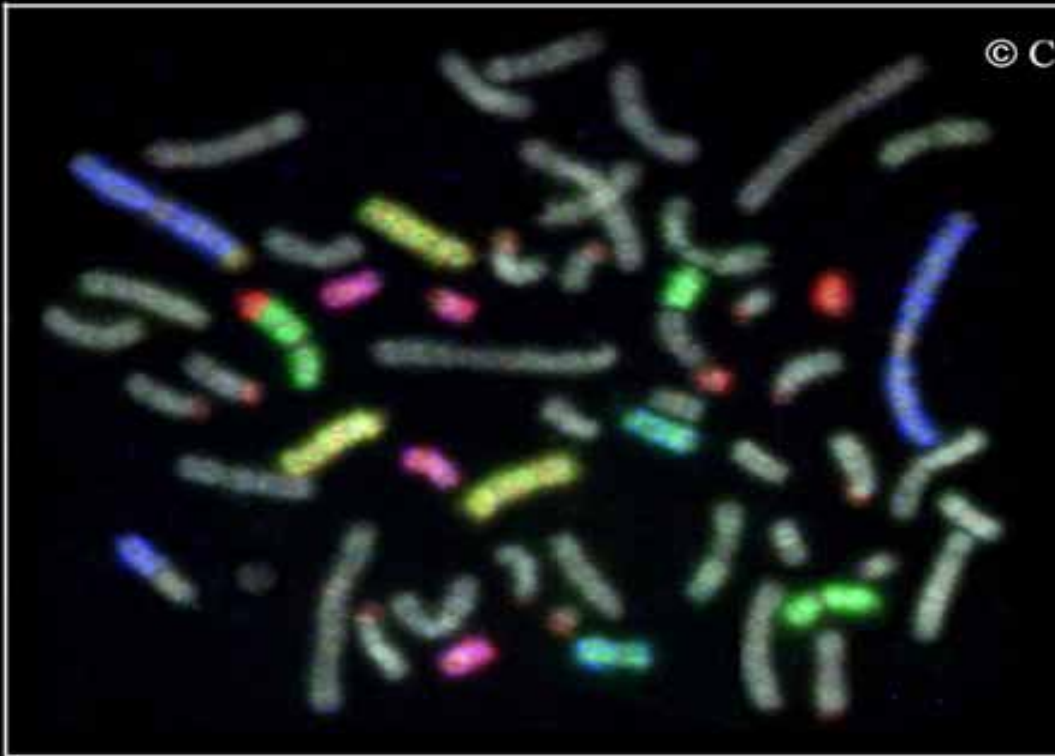


Tritic
wheat
hyk



Human chromosomes

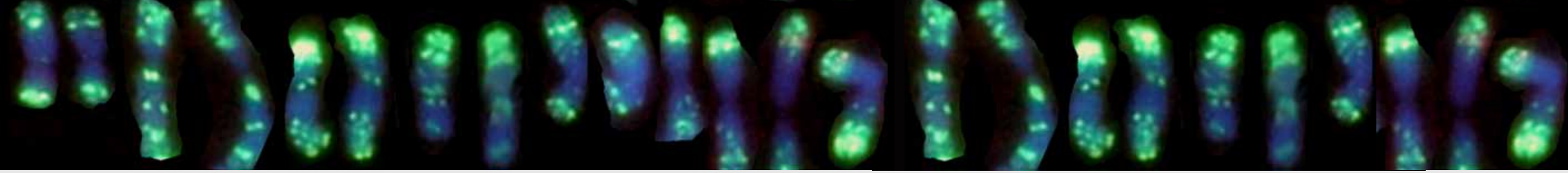
Multi colour FISH with three different fluorochromes



Chromosome	color 1	color 2	color 3	resulting color
22	Red			Red
9		Green		Green
1			Blue	Blue
8	Red	Green		Yellow
17		Green	Blue	Turquoise
19	Red		Blue	Pink
—	Red	Green	Blue	

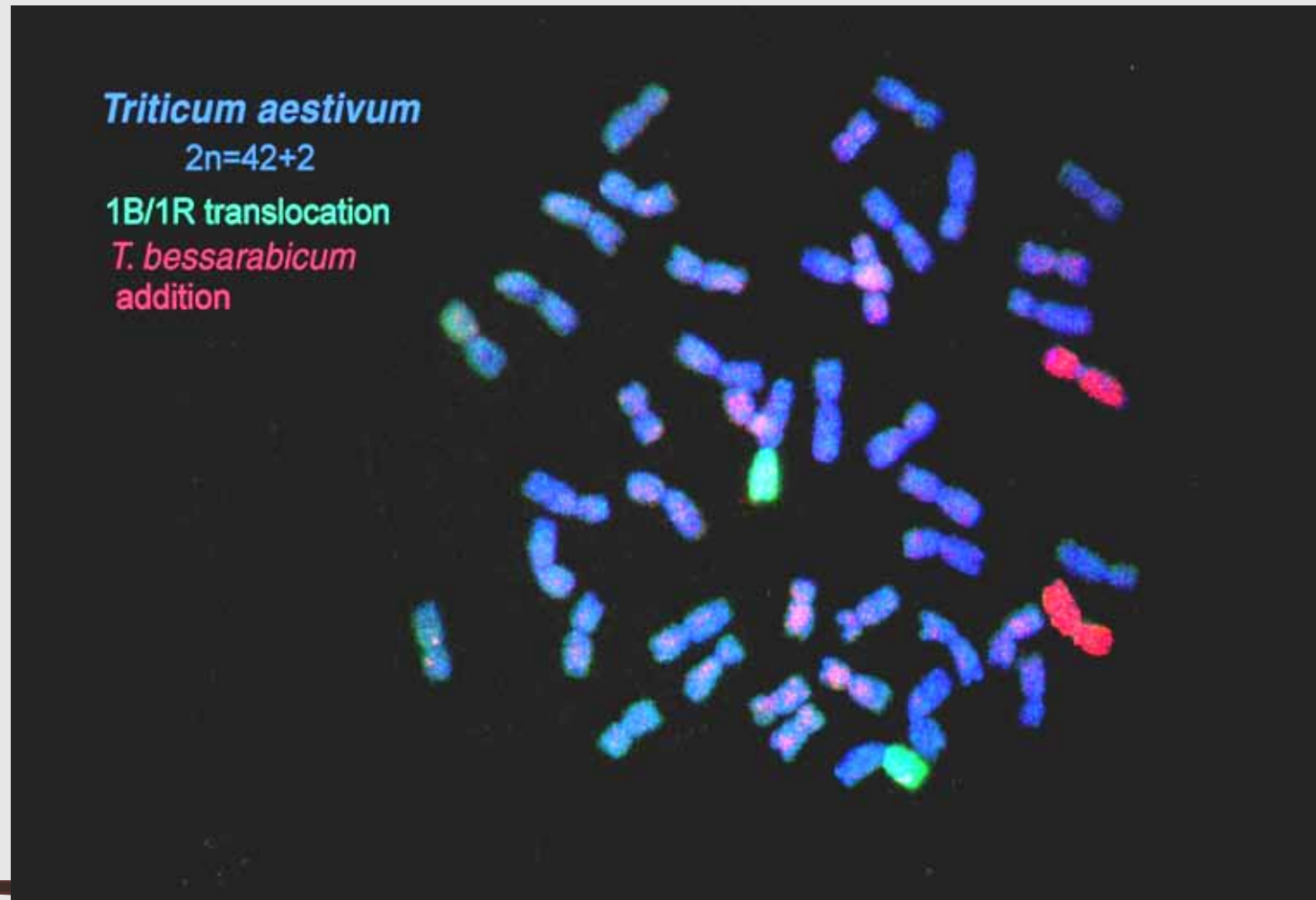


Chromosome painting probes are labelled in Boolean combinations, thus, three fluorochromes would display seven different colours (blue, green, red, for the paints labelled with one fluorochrome and yellow, pink and turquoise for the paints labelled with two fluorochromes. The seventh combination using all three fluorochromes would result in a white colour (not used in this experiment, see insert). The paints are hybridized to chromosomes from a chronic myeloic leukemia (CML) cell line showing various chromosome rearrangements



Total genomic DNA can be used as a probe to distinguish

- Genomes in sexual hybrids
- Alien chromosome introgression



A decorative header image showing a series of chromosomes stained with green and blue fluorescent dyes against a black background.

Breeding strategies

AABBDD x RR

ABDR

Double chromosomes

AABBDDRR

Backcross to wheat

Looses the R chromosomes eventually

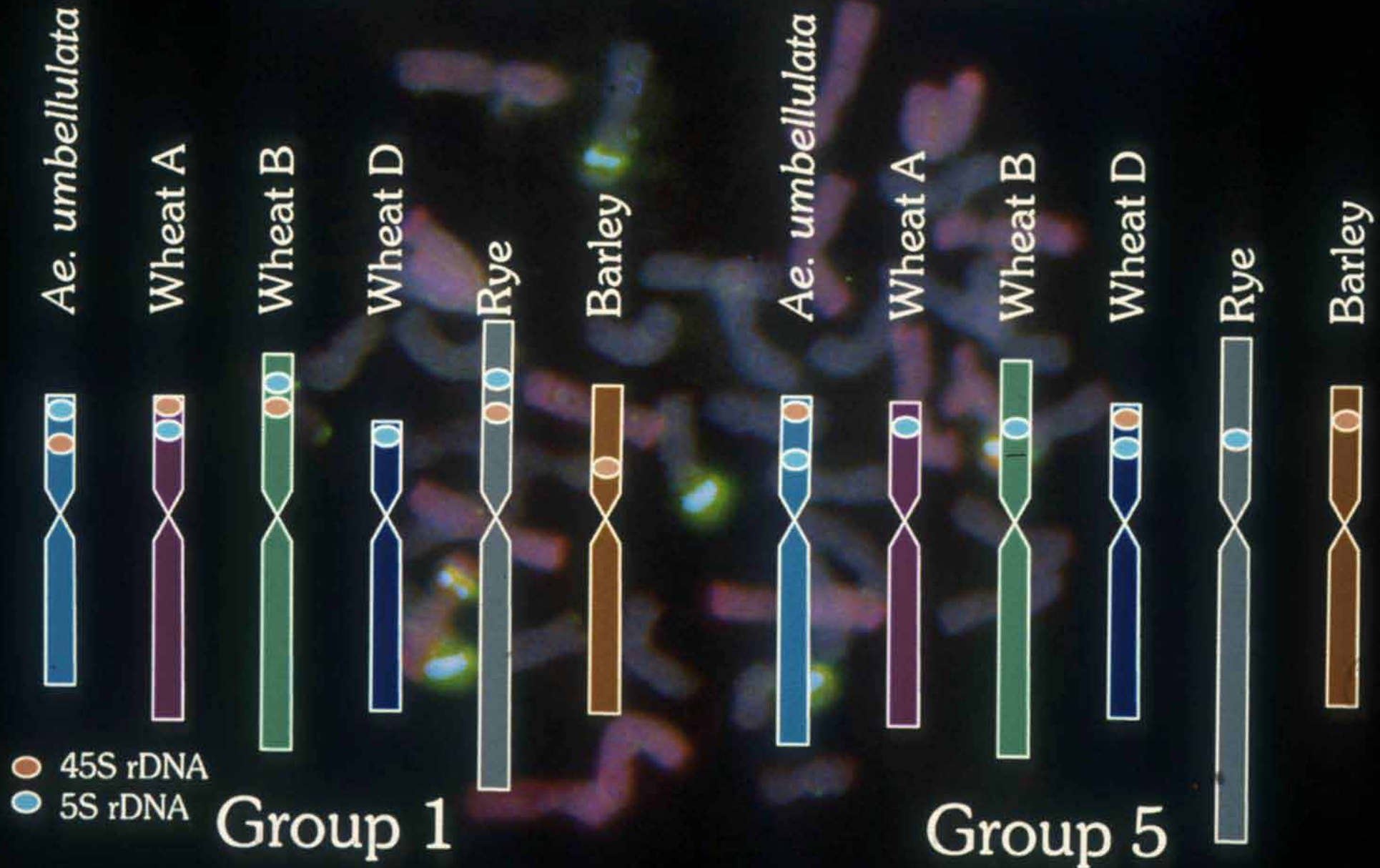
AABBDD+1R

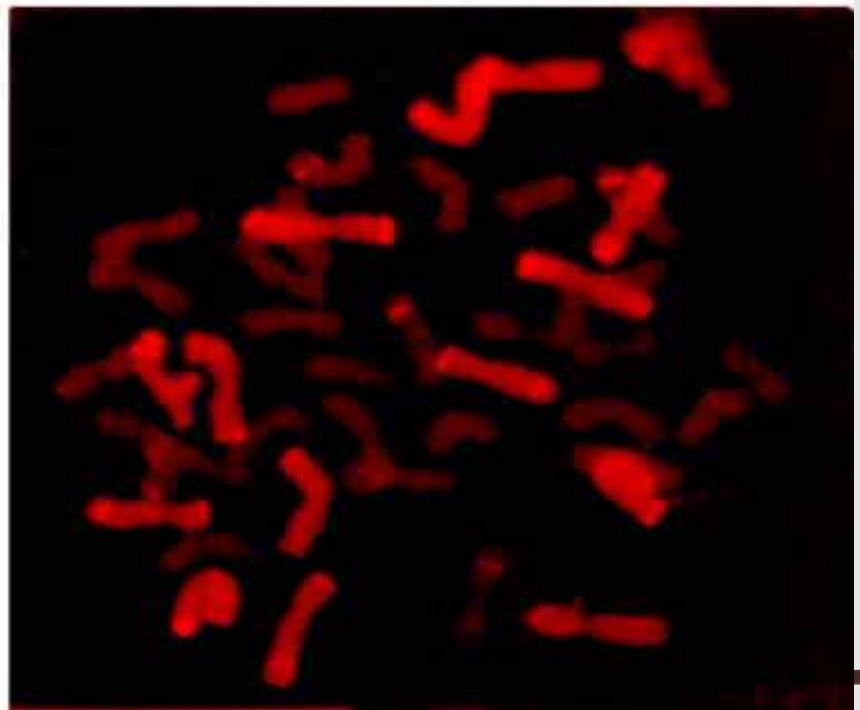
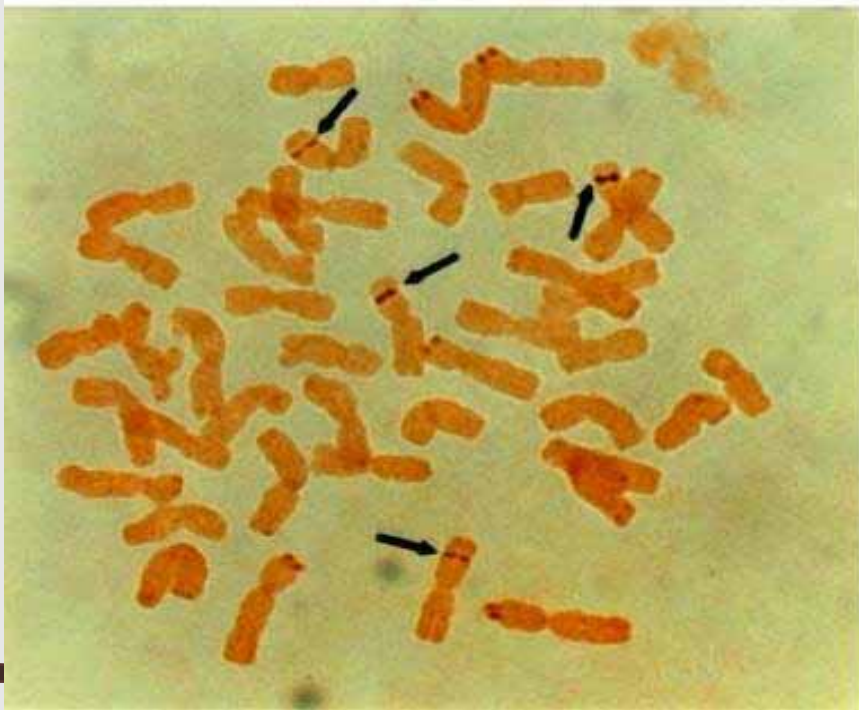
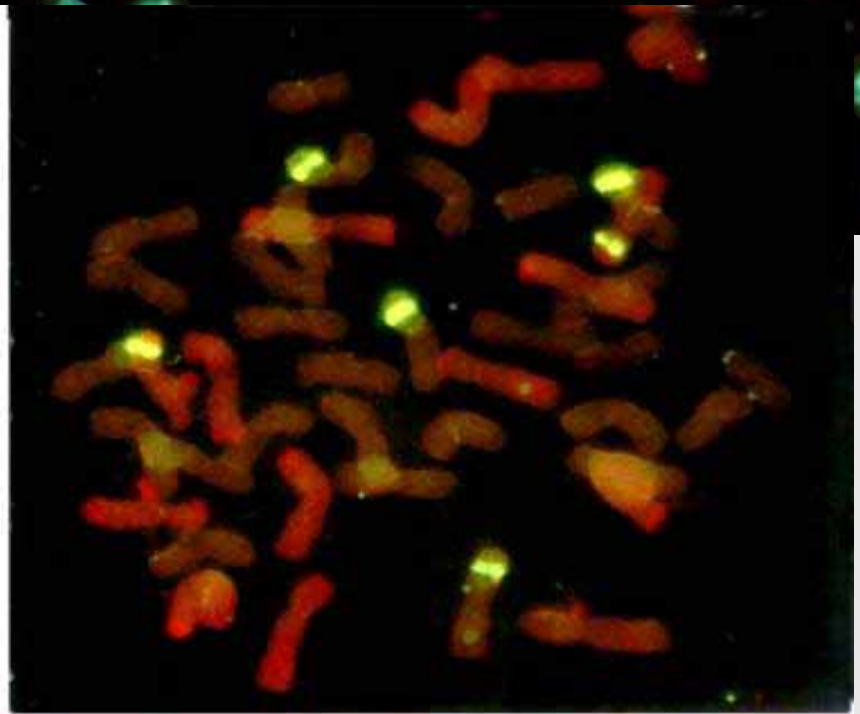
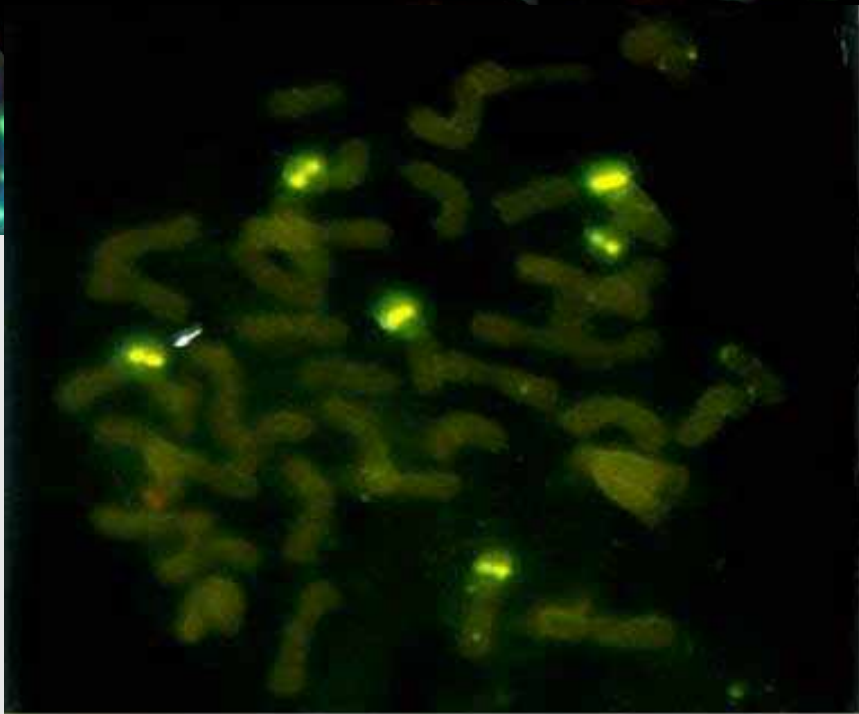
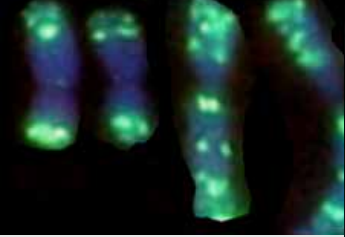
Induce recombination

AABBDD including 1BL/1RS

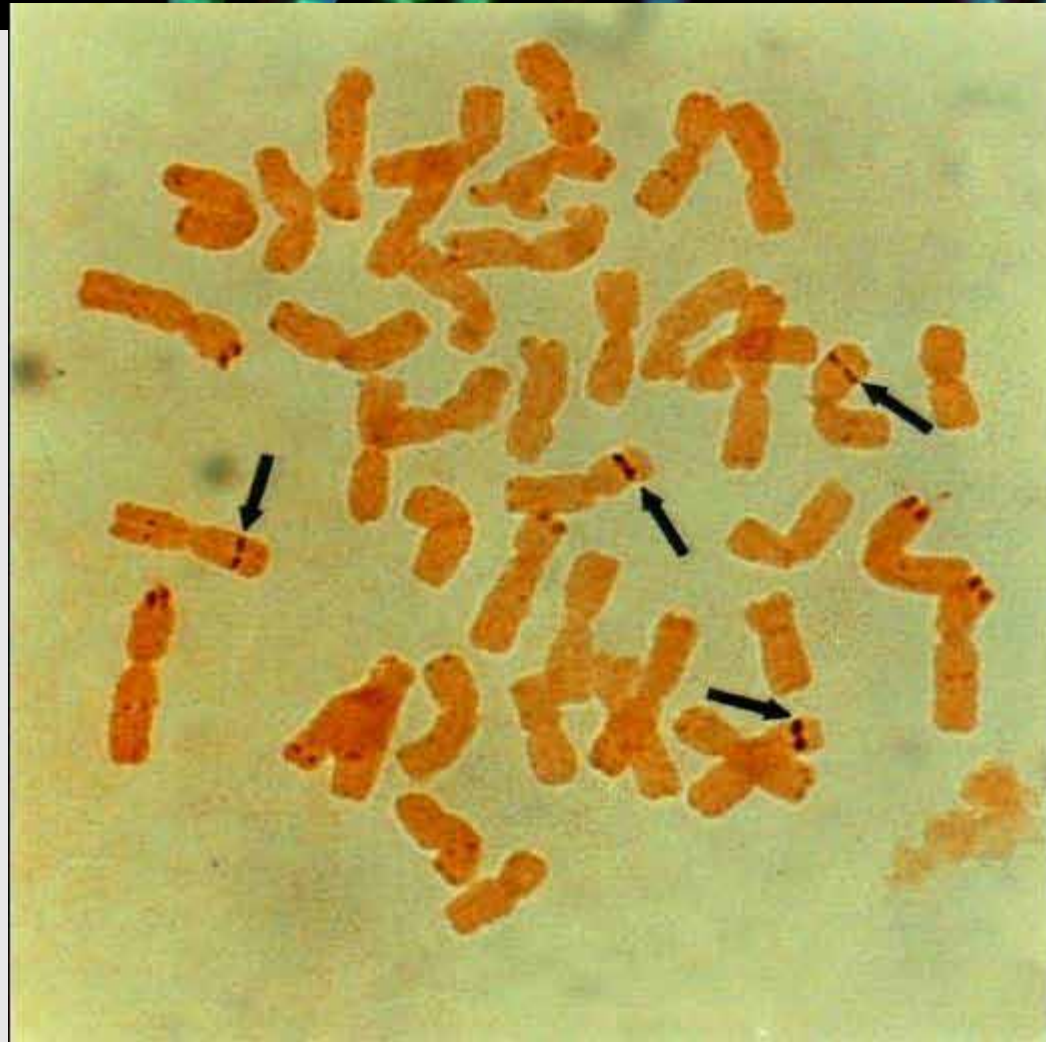
A decorative wavy line at the bottom of the slide, colored in a dark brown or black hue.

rDNA sites in Triticeae Genomes

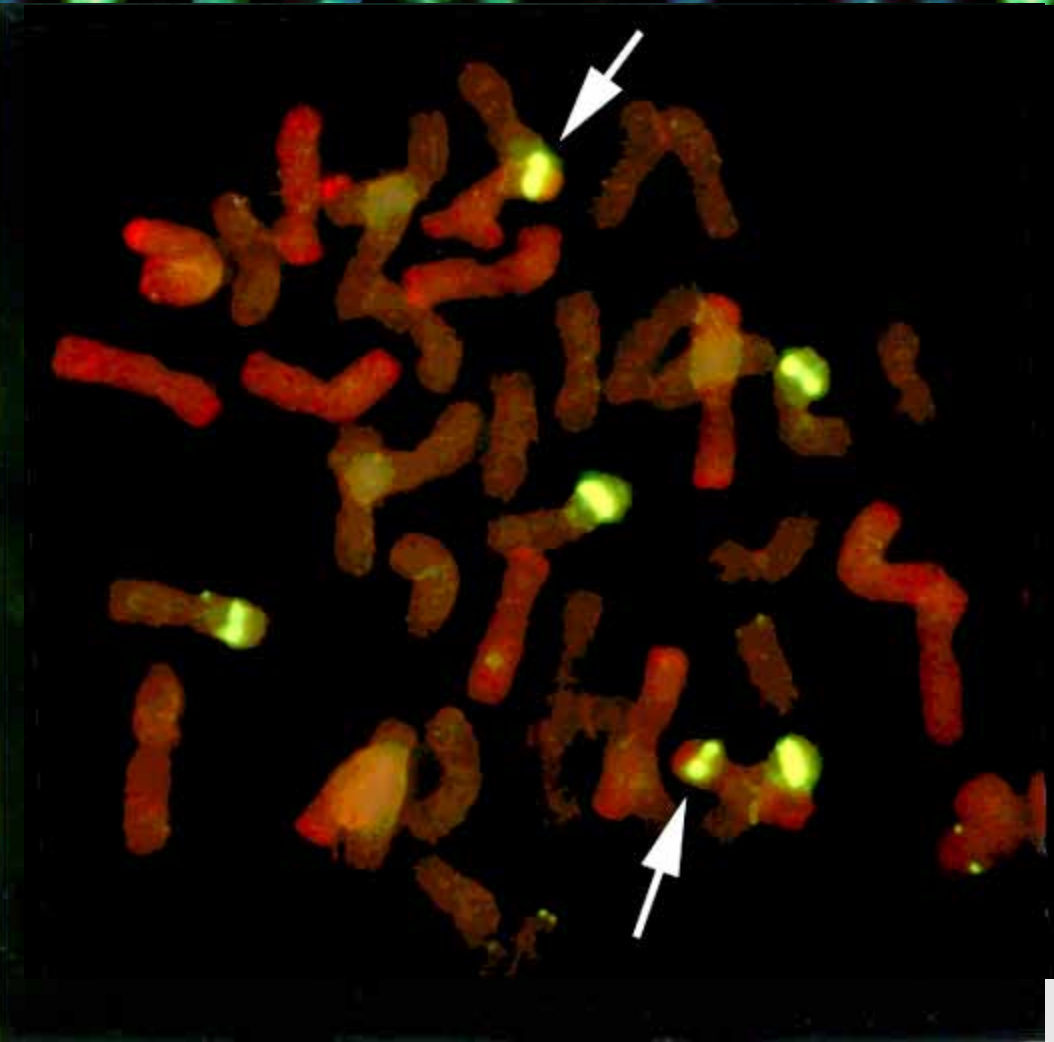




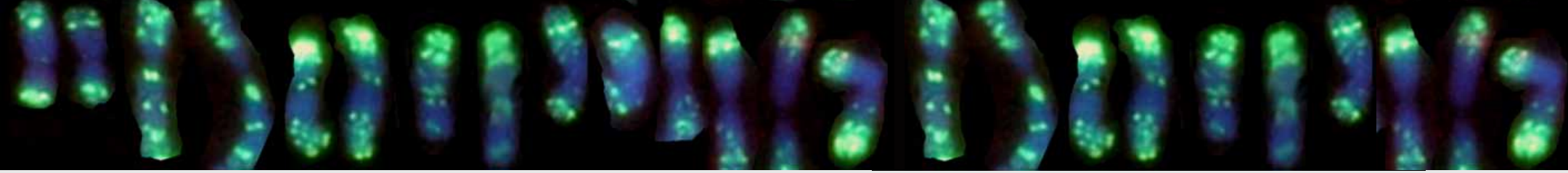
rRNA gene expression in Triticale



Four expression sites

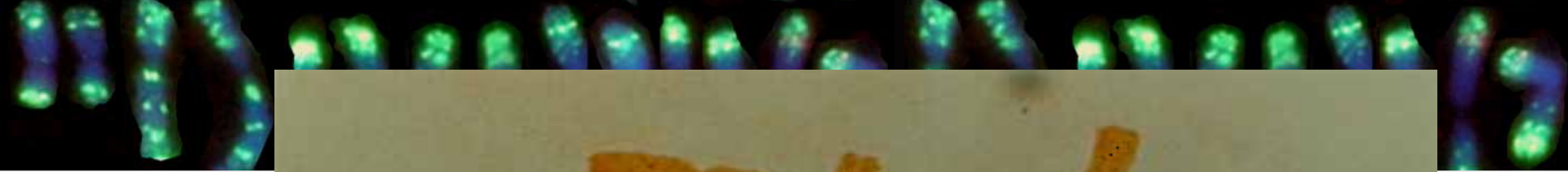


Six gene sites

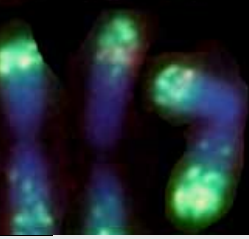


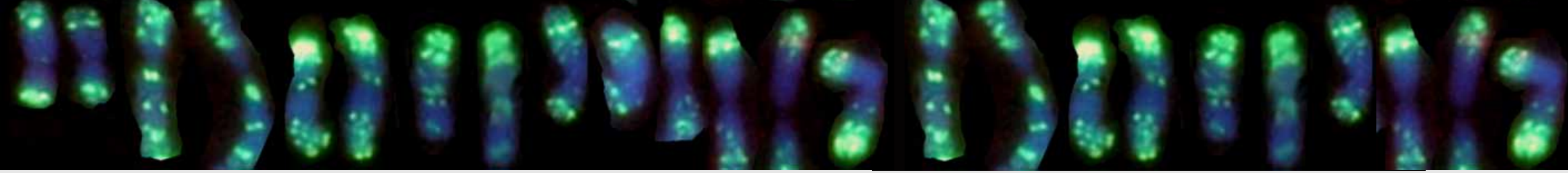
Modification of DNA Methylation

- ☞ 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



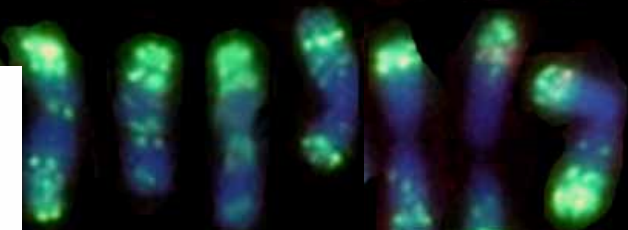
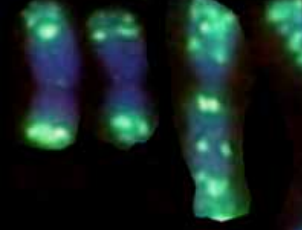
10





DNA analysis by restriction enzymes

- ☛ *ApaI* – *DraI* double digest reveals extra site only in treatments expressing 6 loci
- ☛ At least one restriction site is altered
- ☛ Correlates exactly with sequence analysis

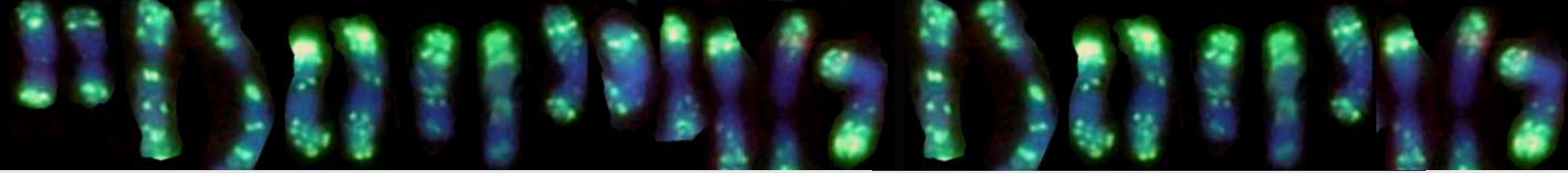


rDNA methylation
Probe

wheat rDNA		rye rDNA spacer	
Treatment			
water	AZC	water	AZC

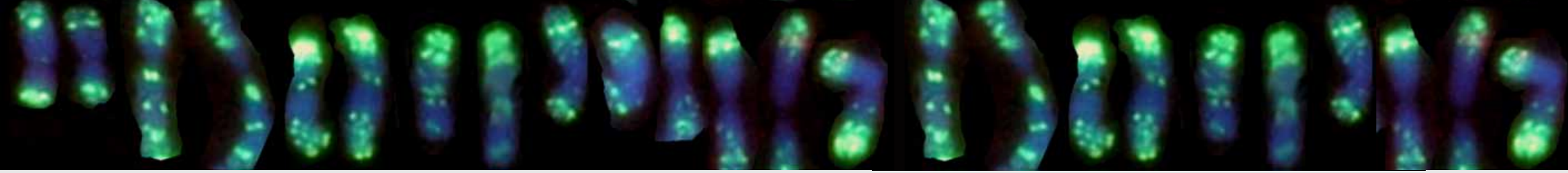


lambda	Apal	DraI	double	Apal	DraI	double
lambda	Apal	DraI	double	Apal	DraI	double



Modification of rDNA Methylation

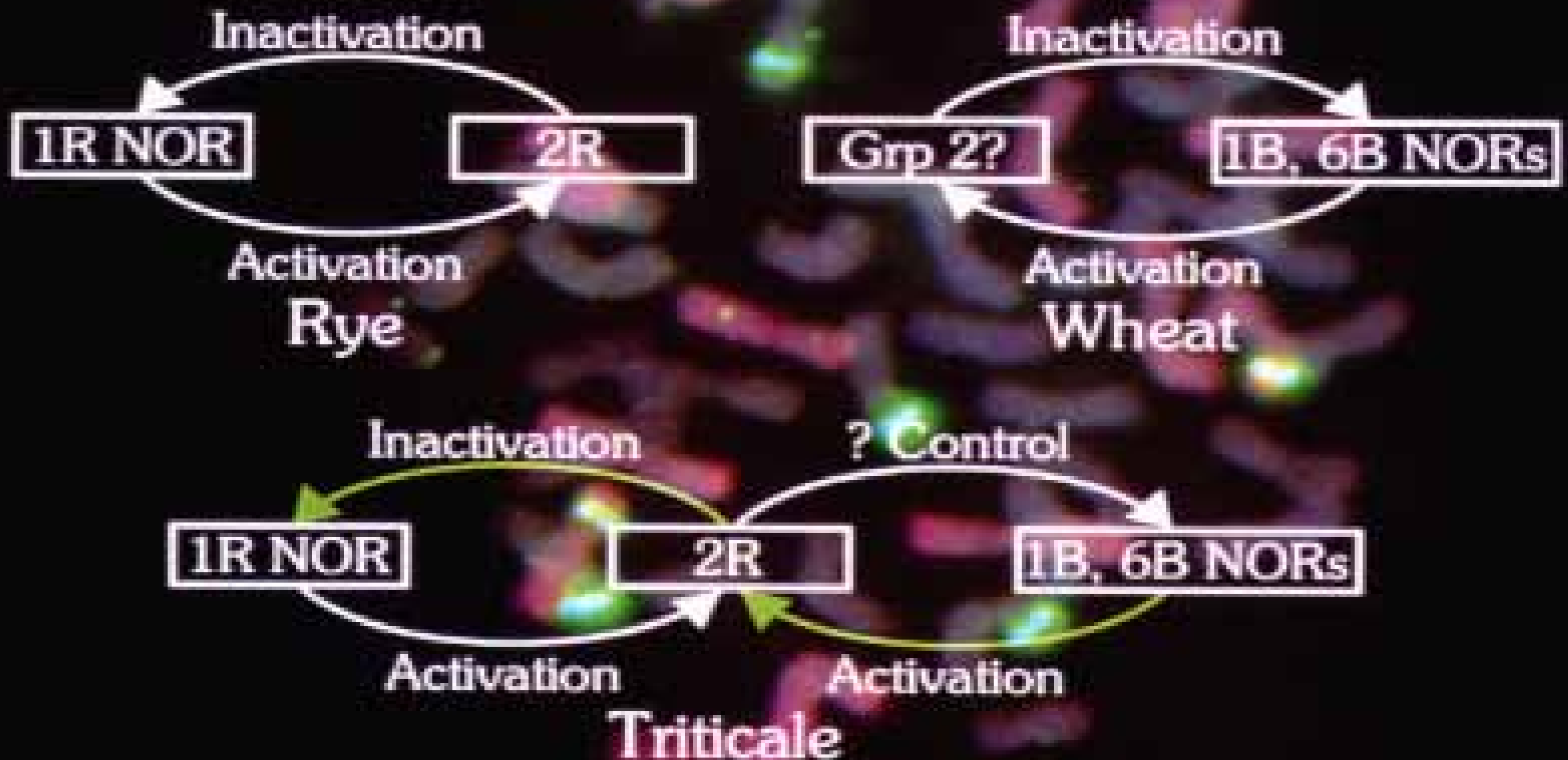
- ☛ Stability of methylation
- ☛ Seedling treatment – all 6 active for life
- ☛ Embryo treatment:
 - ☛ First 7 days – only wheat-origin active
 - ☛ After 7 days – rye and wheat-origin active



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

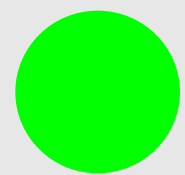




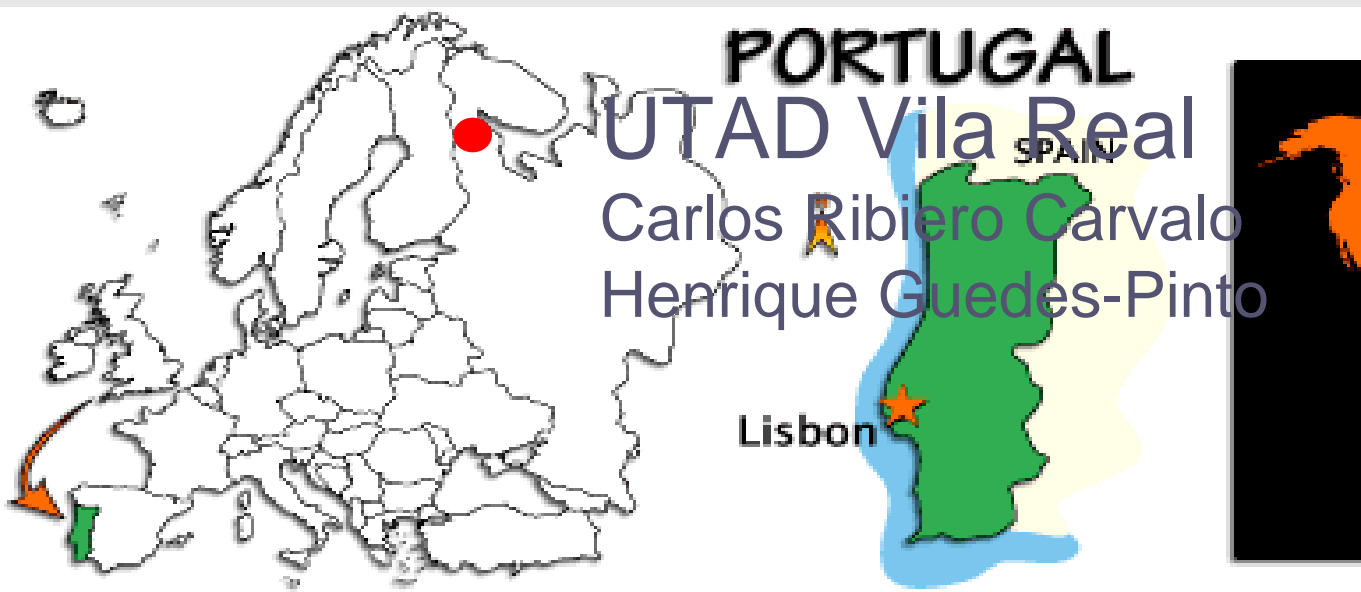


Diversity from a landrace

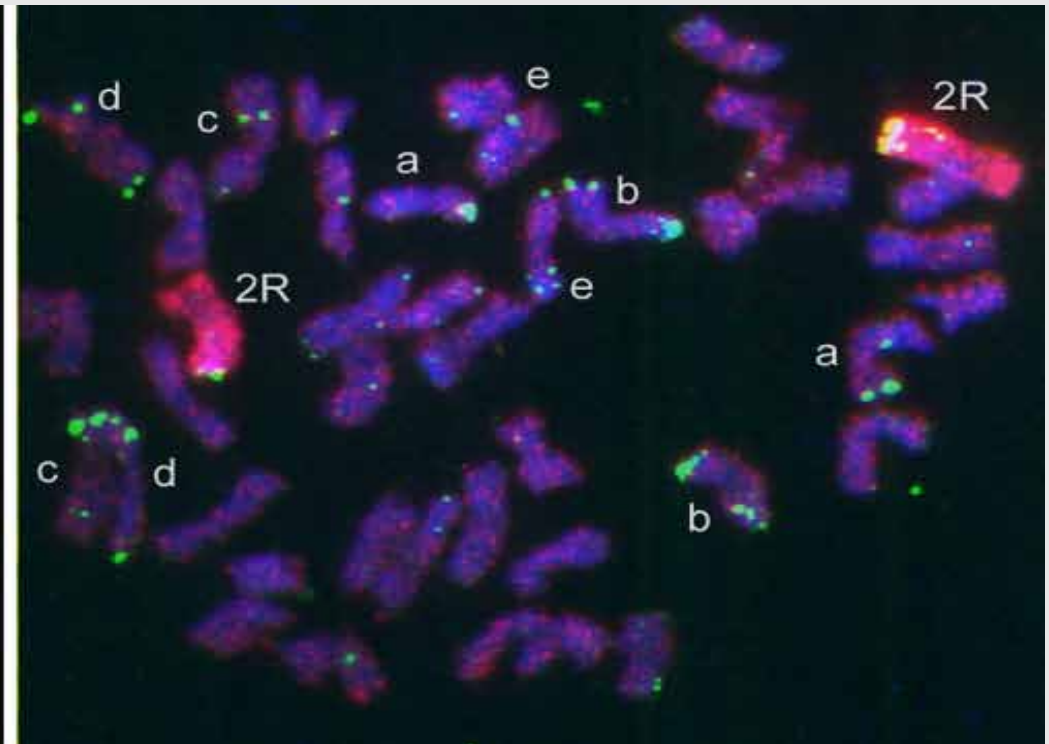
Barbela - a wheat landrace
with rye characters



Barbela wheat
collections

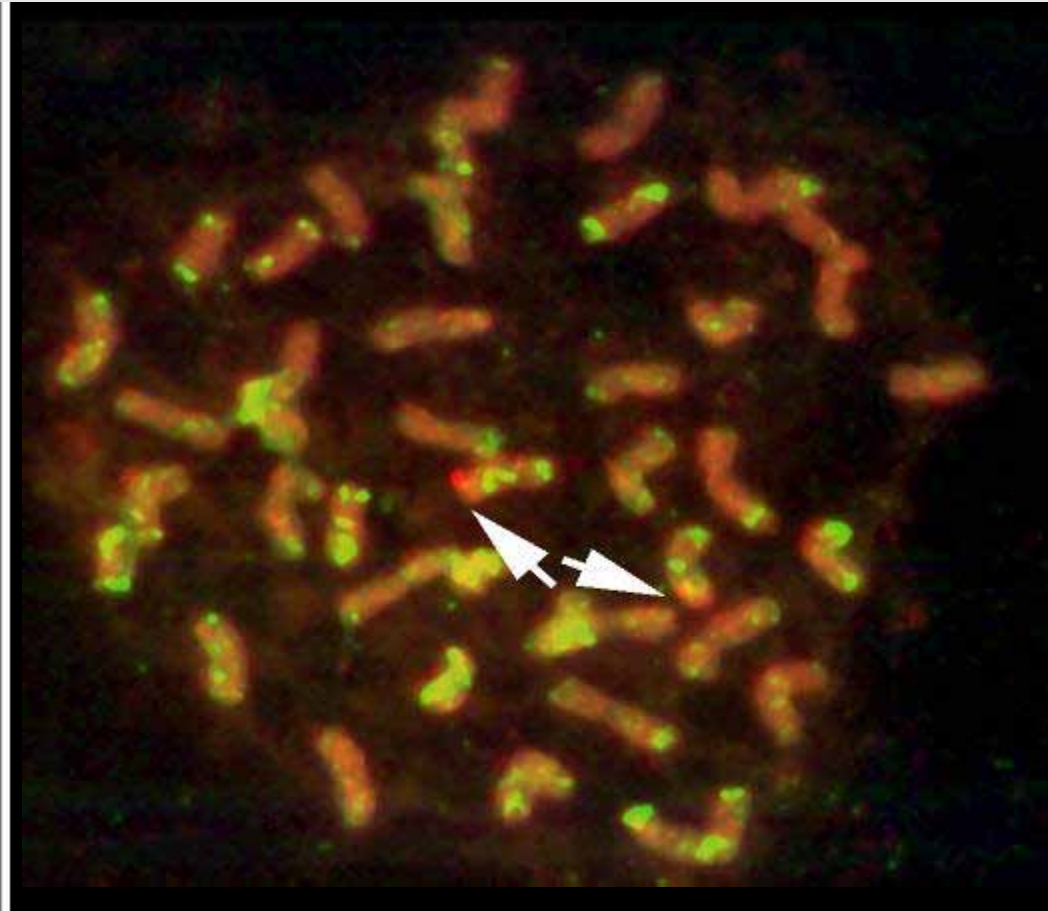
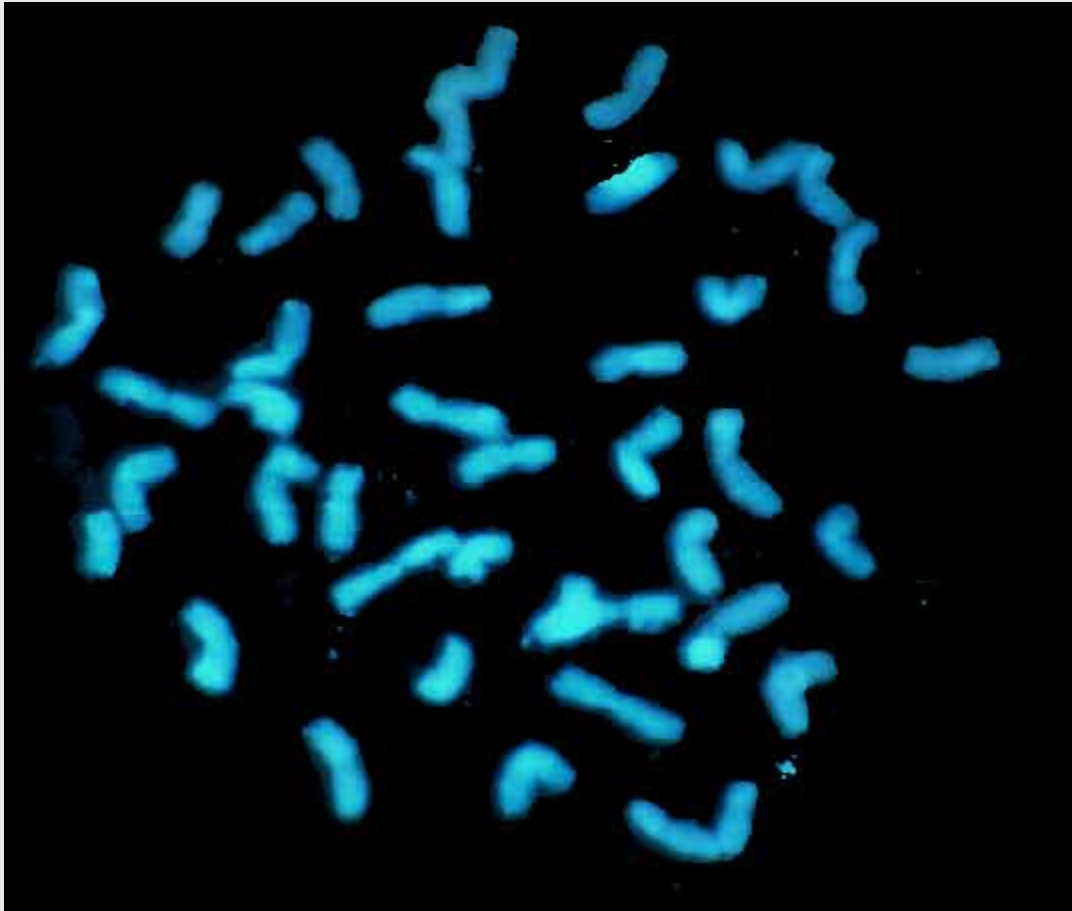


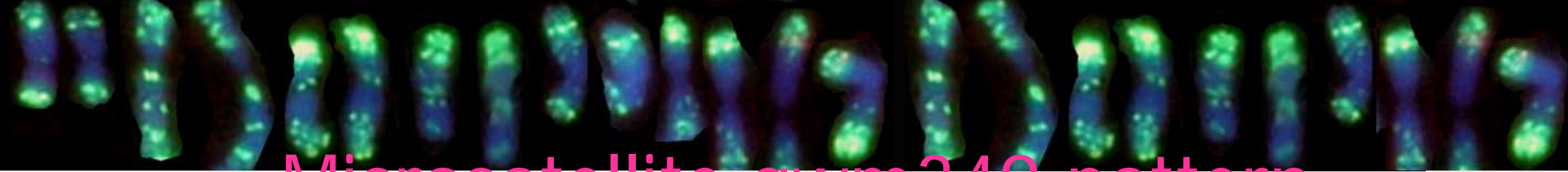
2D-2R substitution - *in situ* hybridization



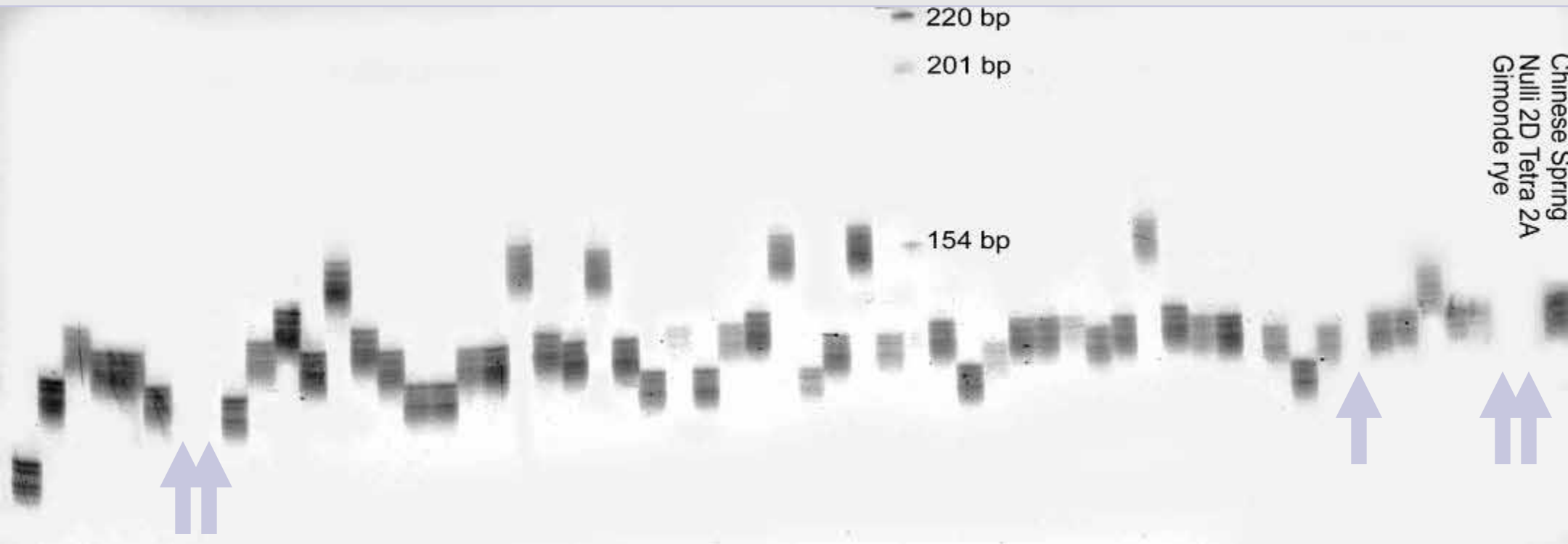
Total genomic rye DNA shows rye-origin chromosomes
dpTa1 tandem repeat allows chromosome identification

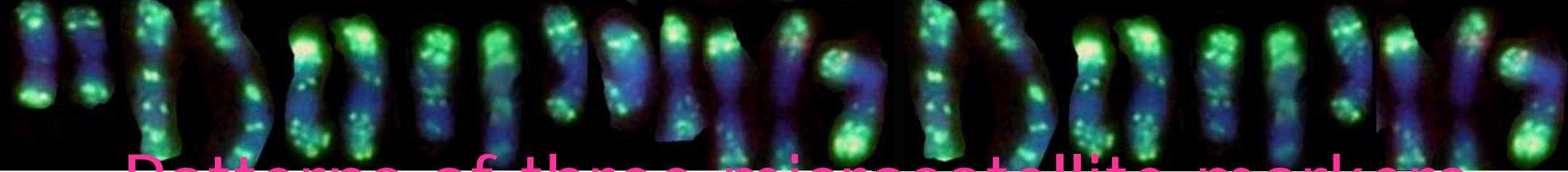
In situ hybridization to show rye chromosome segments in Barbela



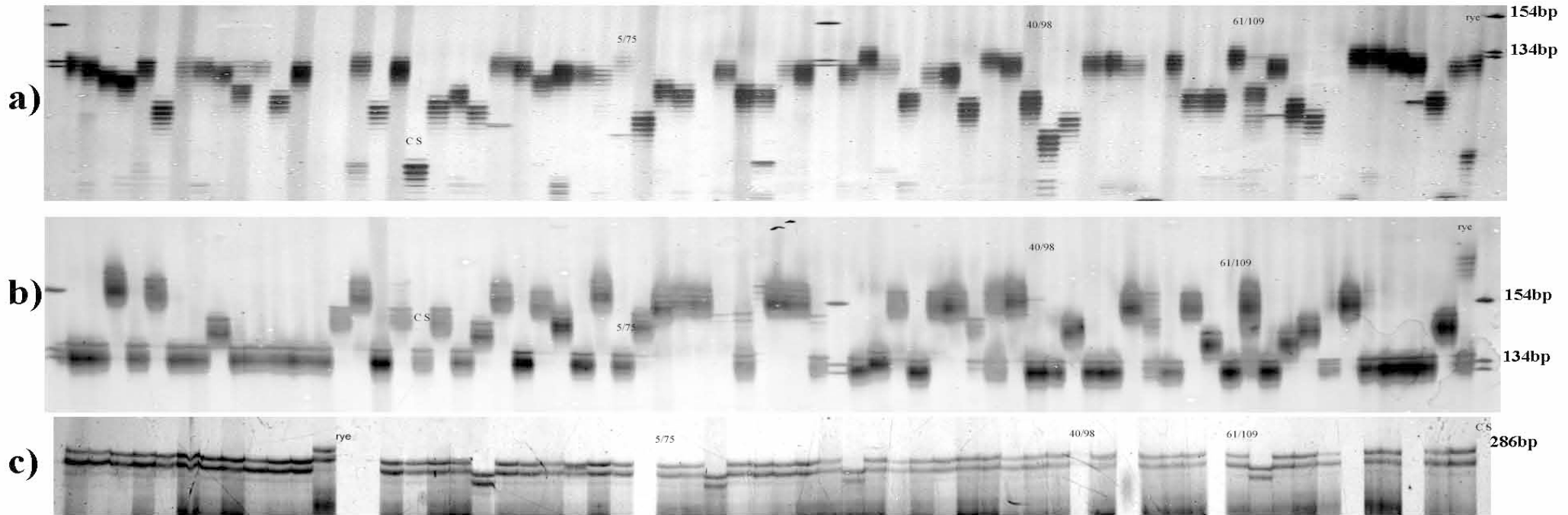


Microsatellite gwm349 pattern





Patterns of three microsatellite markers



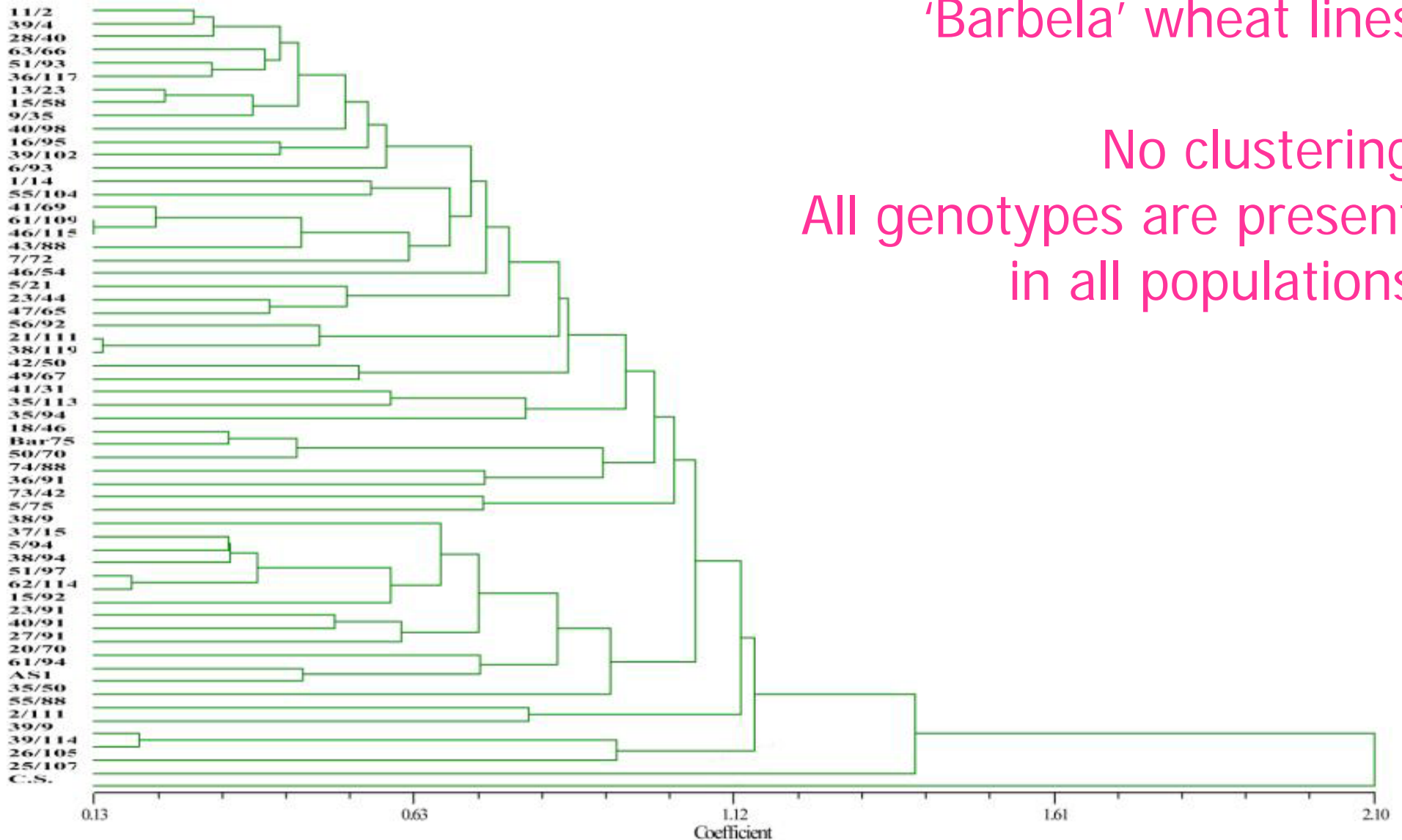
a - gwm276-7AL

b - gwm645-3DL

c - psp3000-1B

Dendrogram distinguishing all 'Barbela' wheat lines

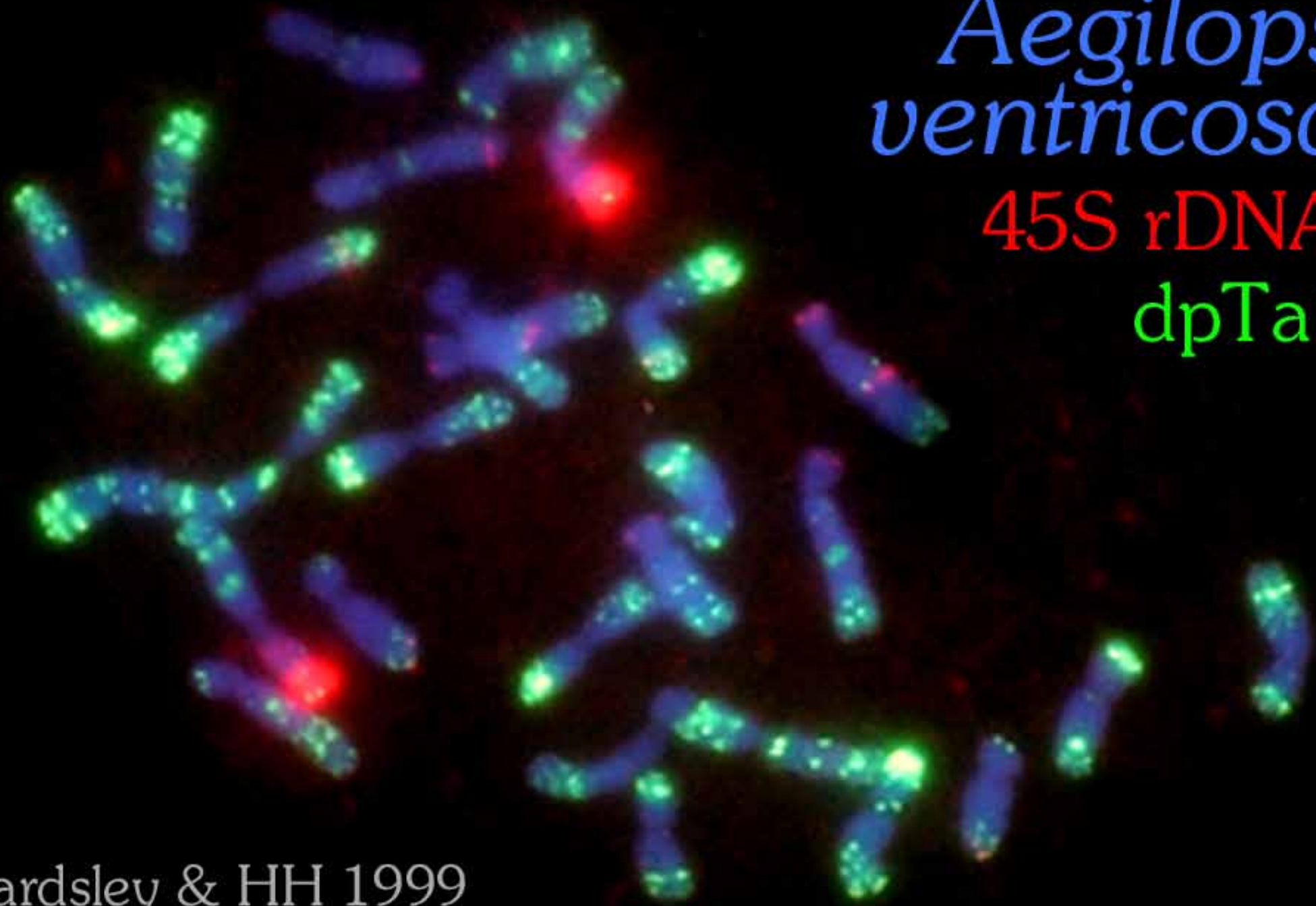
No clustering
All genotypes are present
in all populations

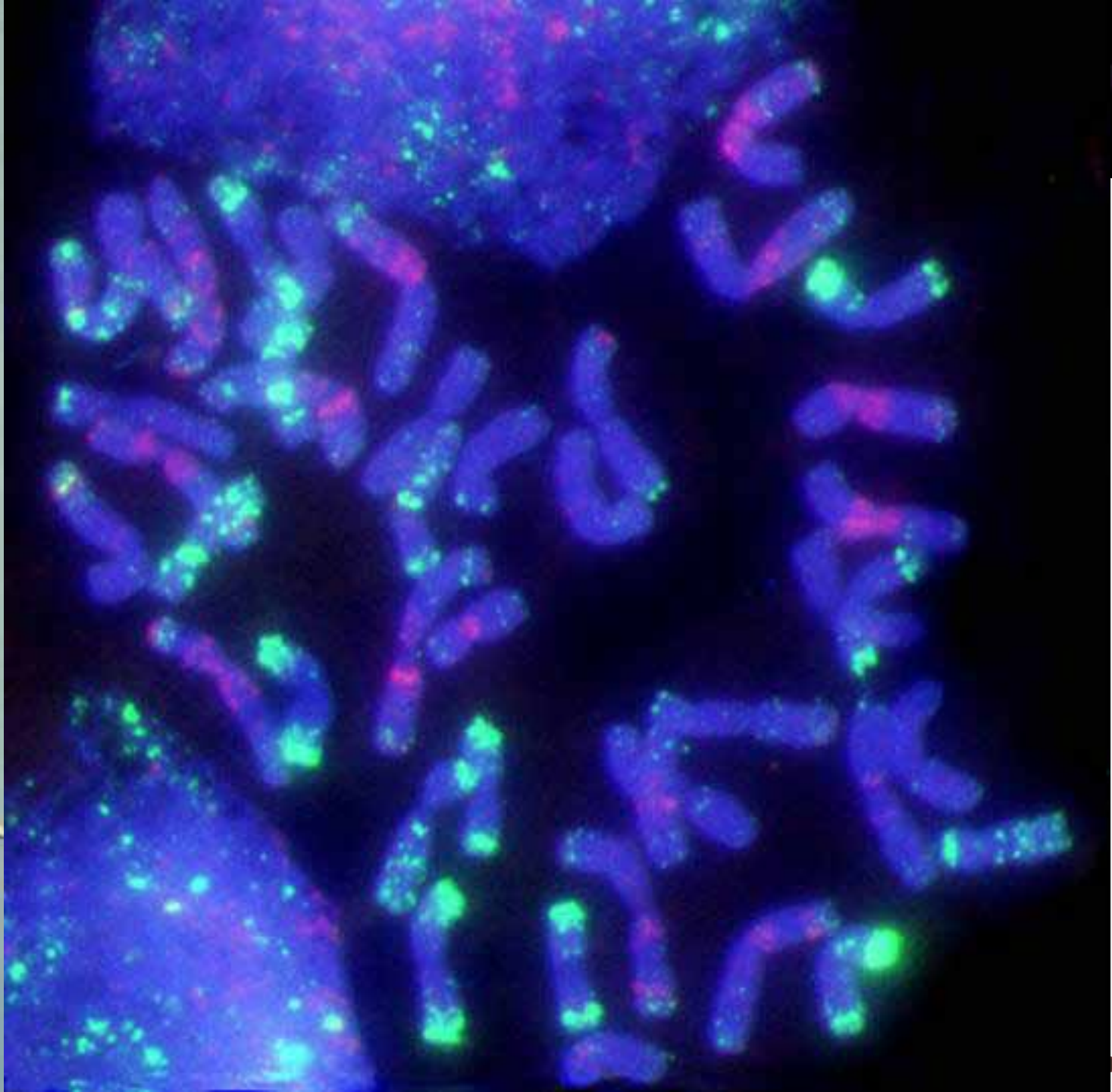


*Aegilops
ventricosa*

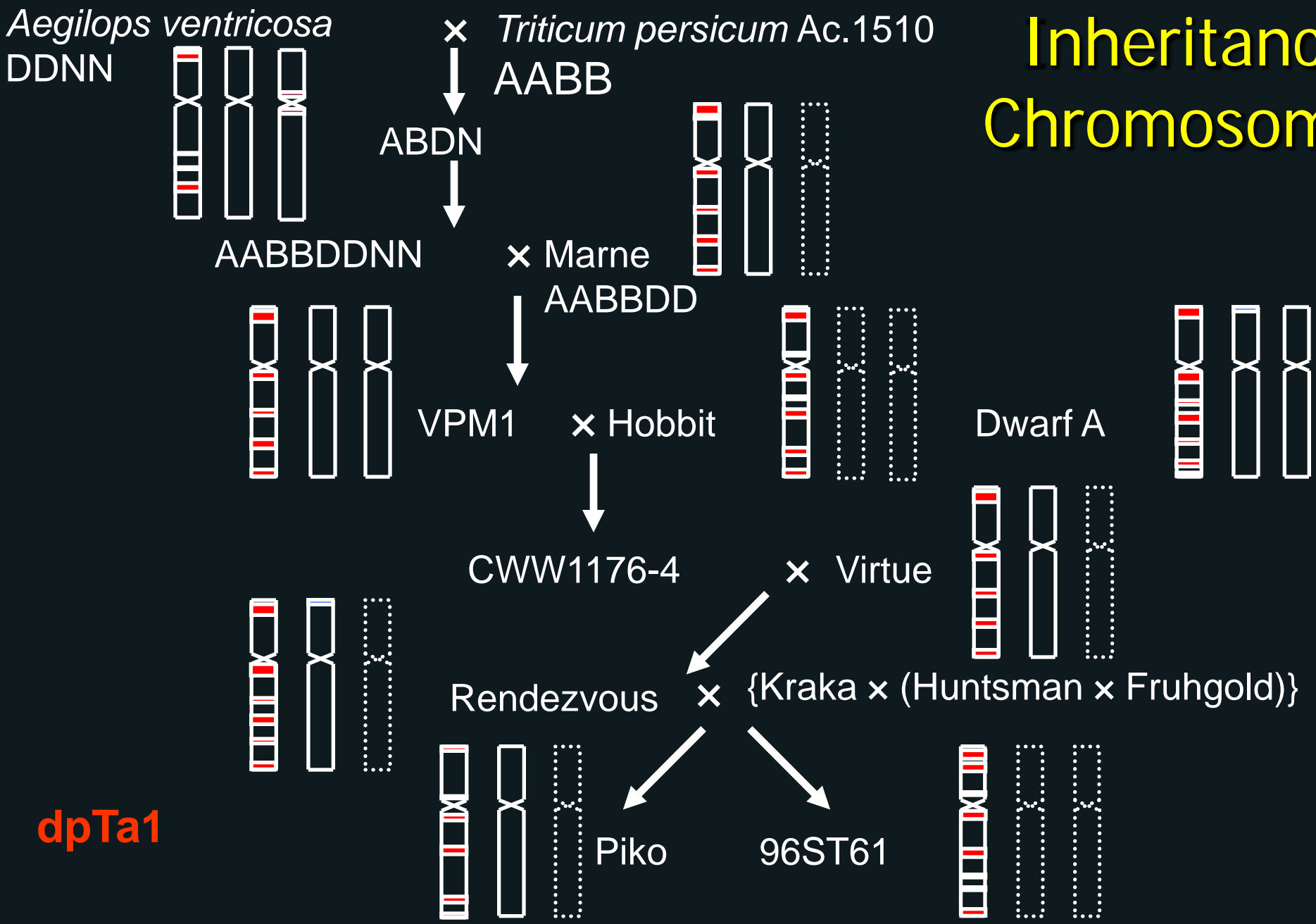
45S rDNA

dpTa1

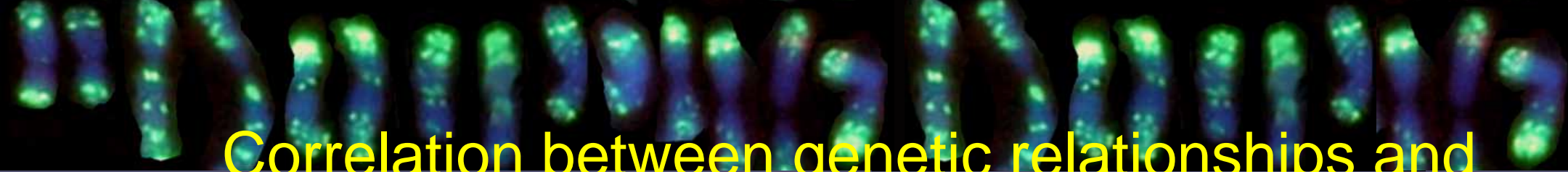




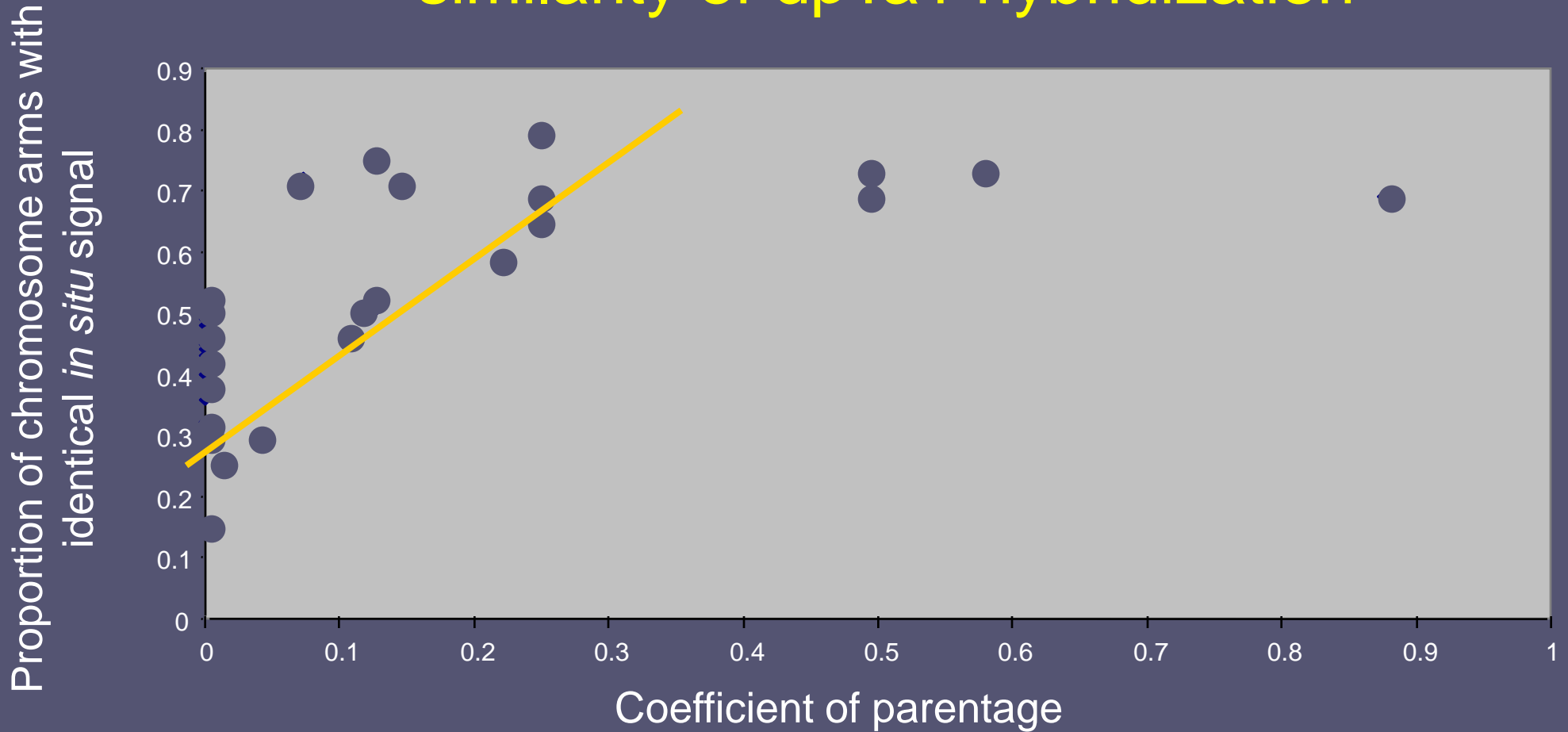
Inheritance of Chromosome 5D



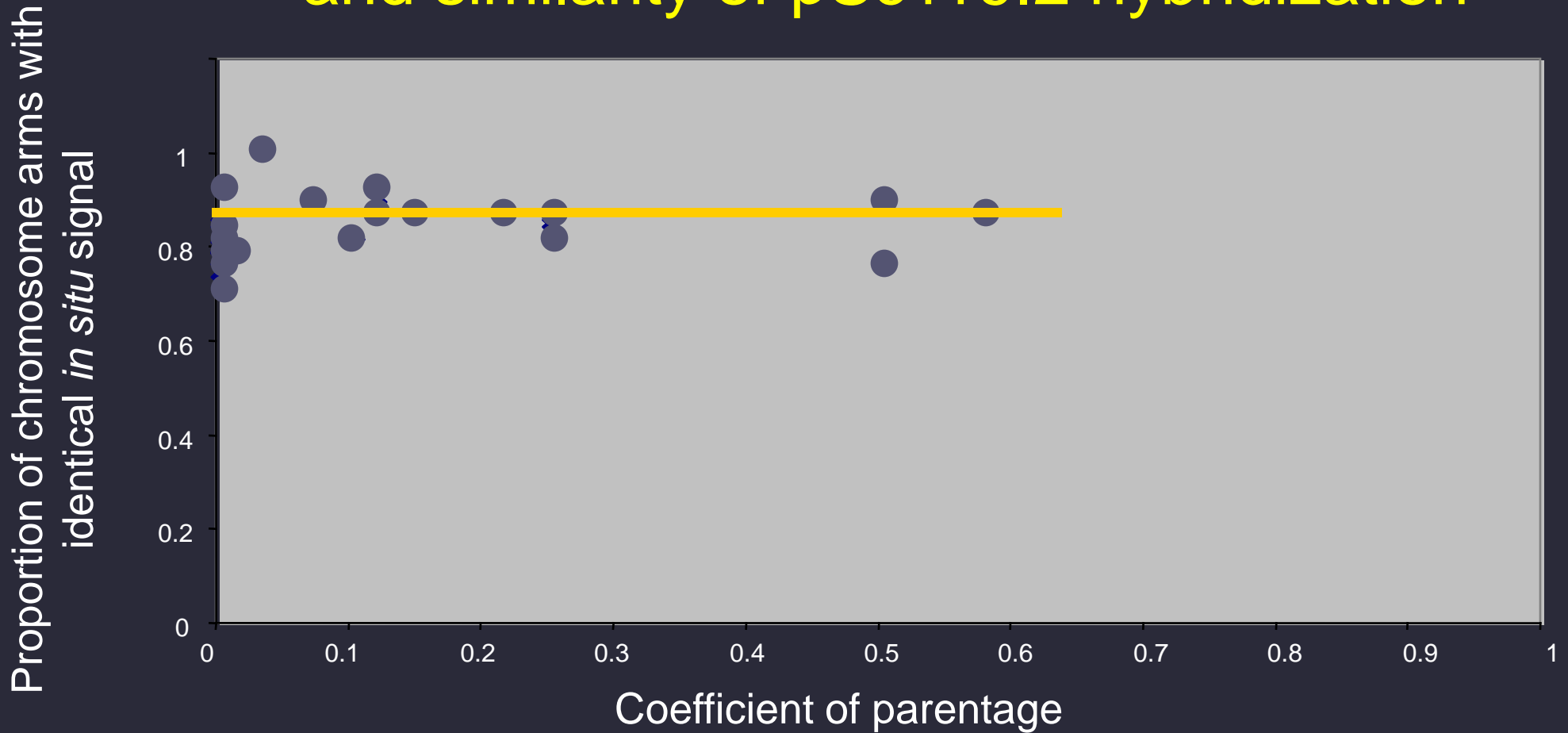
dpTa1



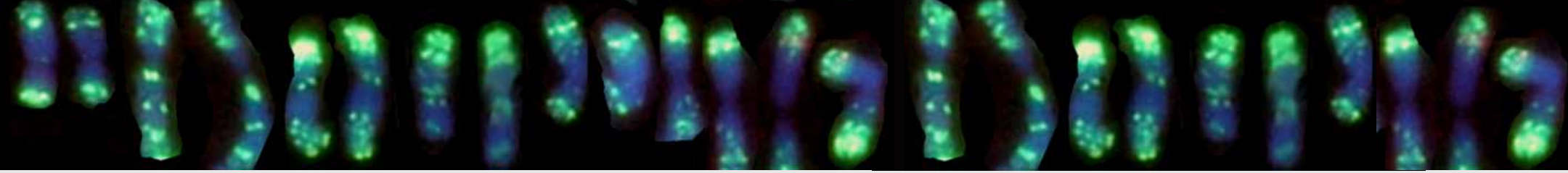
Correlation between genetic relationships and similarity of dpTa1 hybridization



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







Aegilops tauschii (D genome donor) in Iran

57 accessions collected

● *ssp. tauschii*

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



Aegilops tauschii in Iran

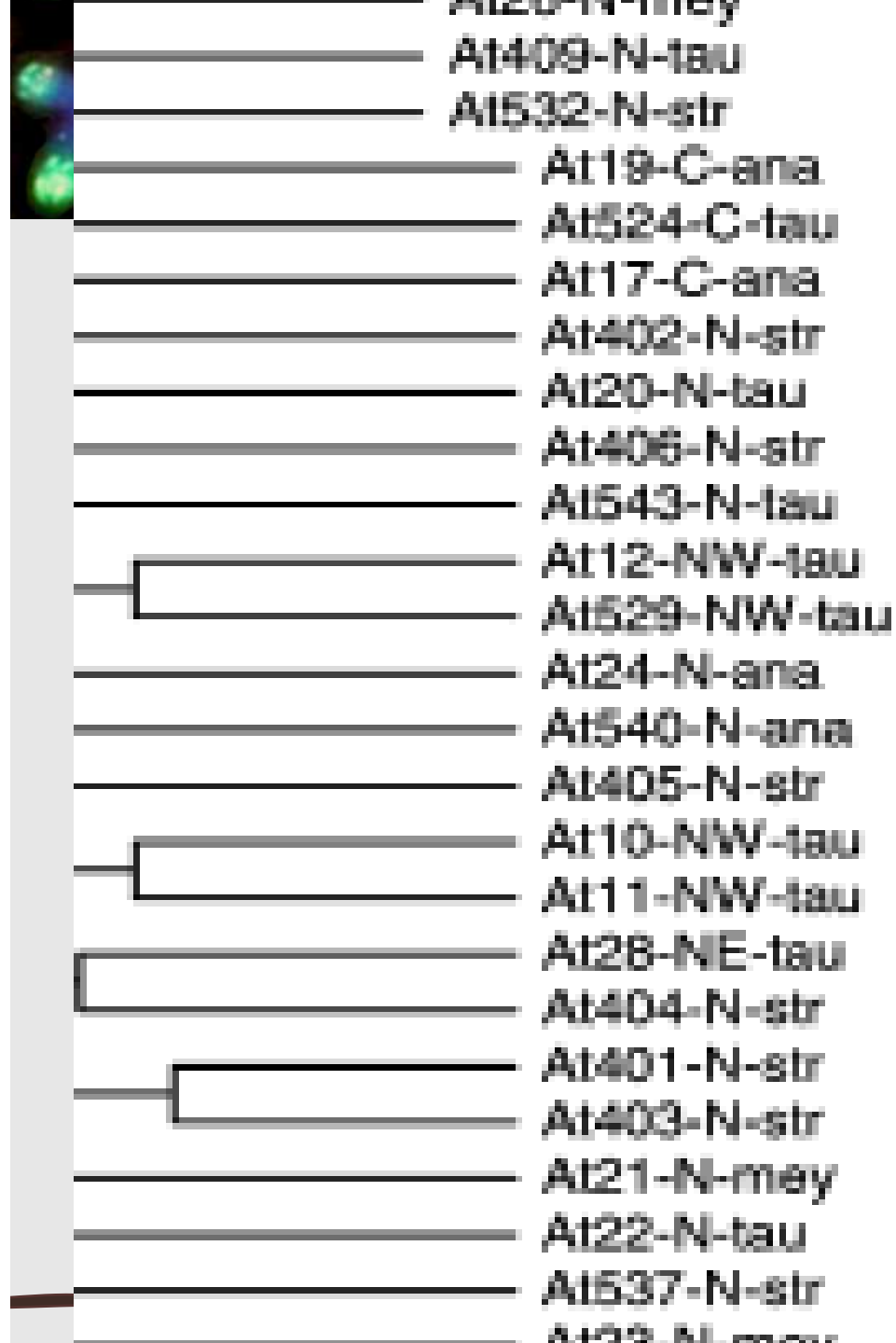
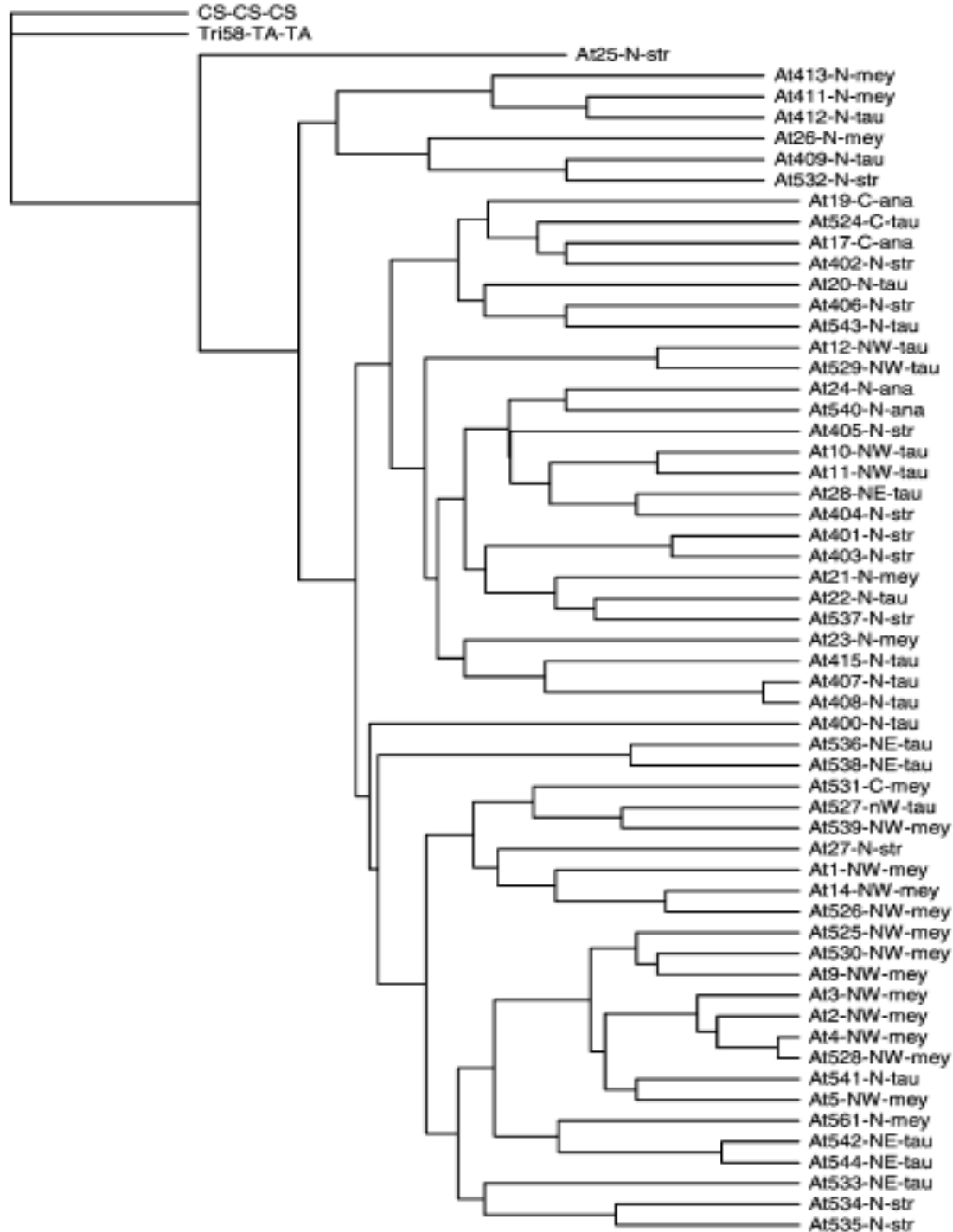
9 SSR markers

66 alleles

no markers were characteristic for taxa or geographical origin

high gene flow between the subspecies and varieties

Hojjatollah Saeidi, Mohammad Reza Rahiminejad, Sadeq Vallian and Pat Heslop-Harrison

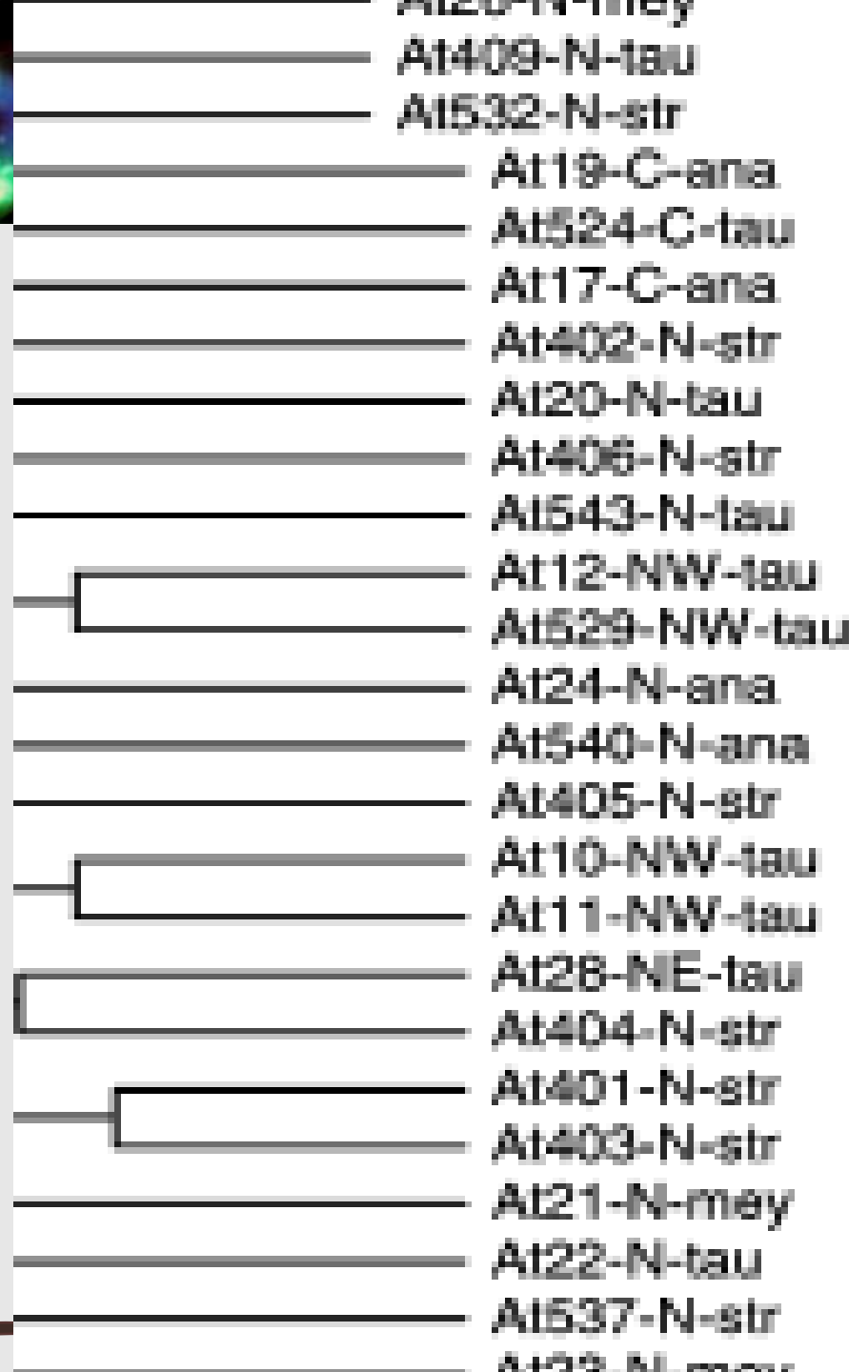




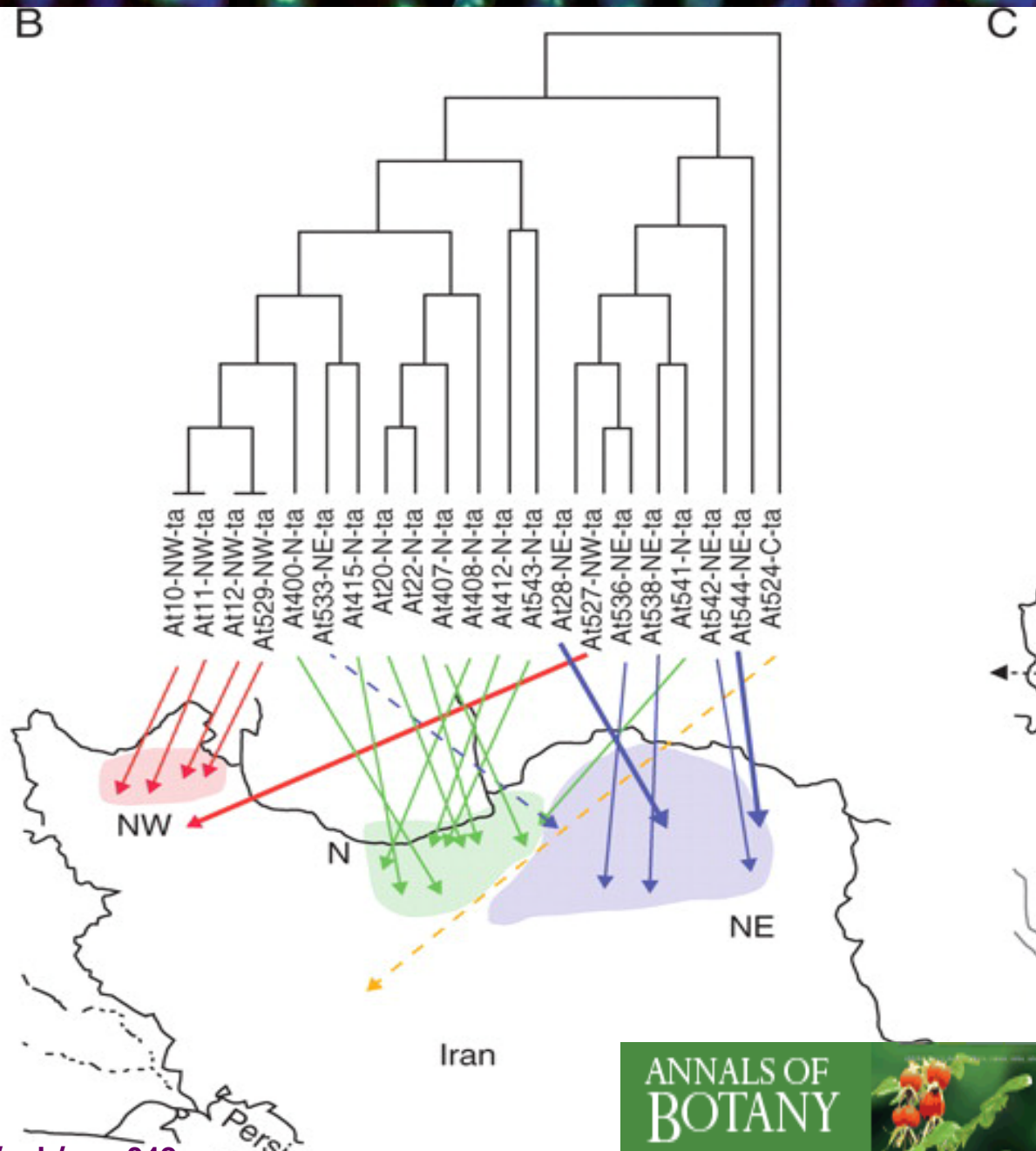
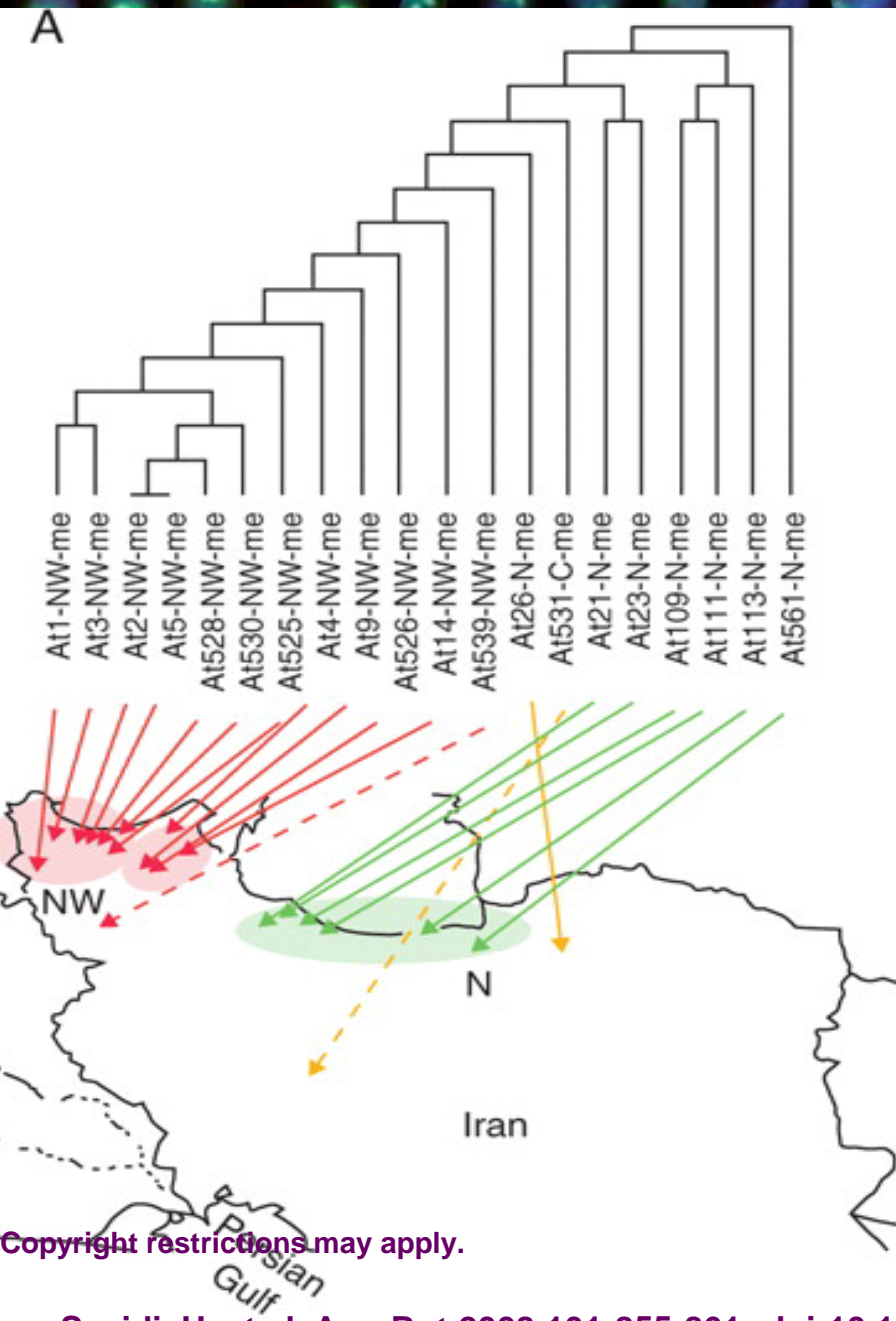
Diversity in D genome

- 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

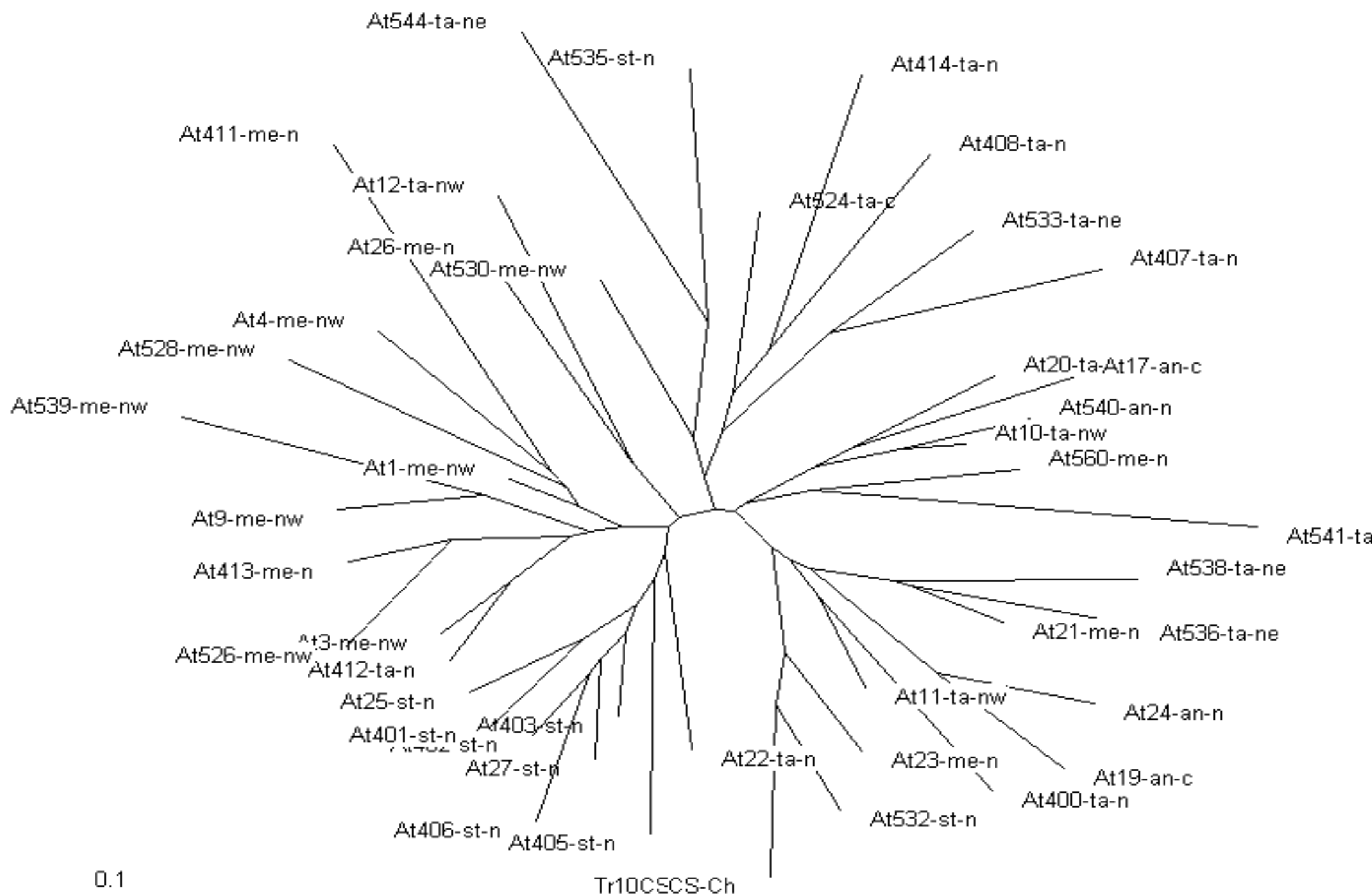
Saeidi, HH et al. Genet Resources & Crop Evolution 2005



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



Copyright restrictions may apply.



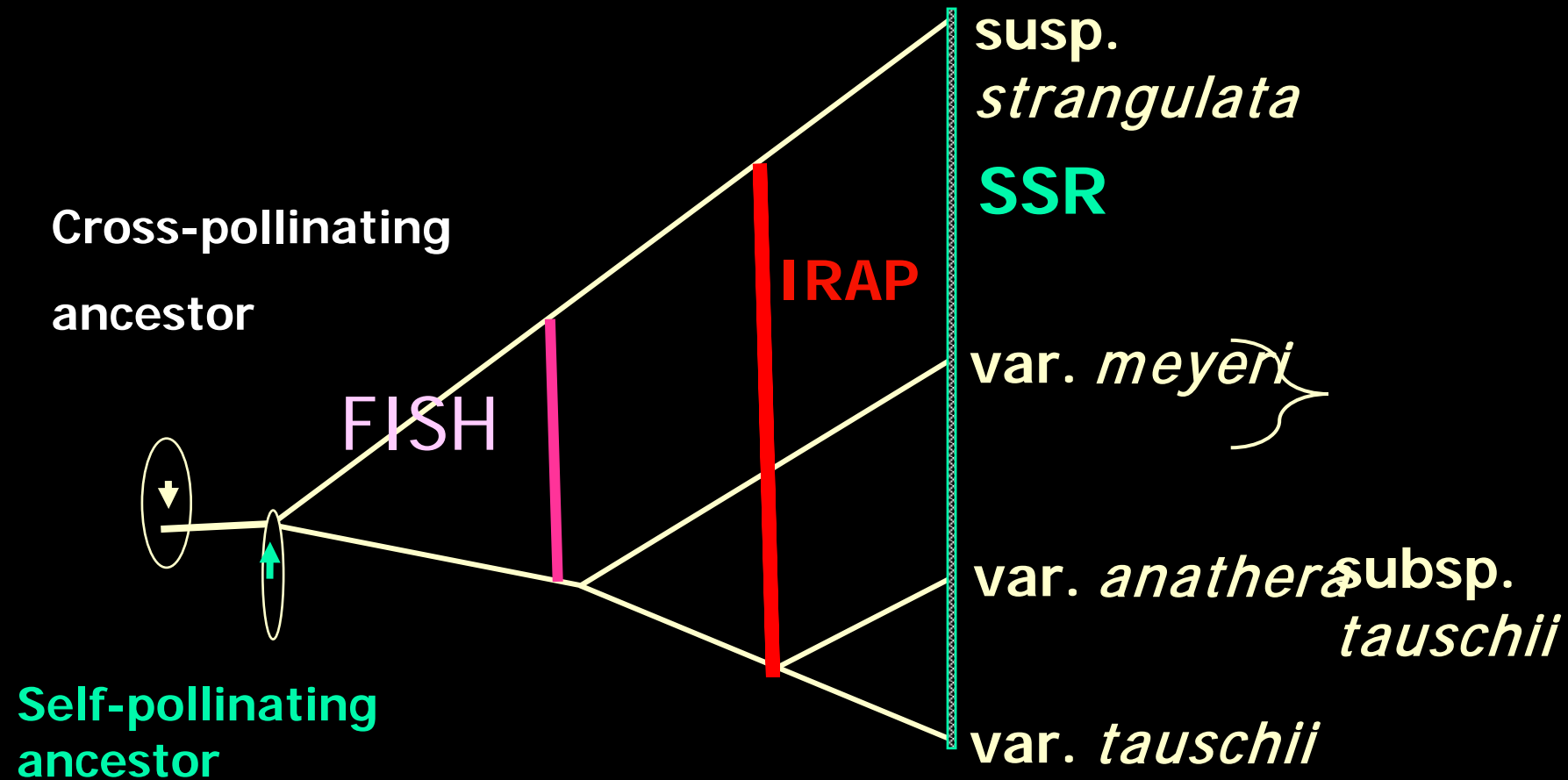
Demonstration of the direction of distribution (phylogeography) even over short geographic distances

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)

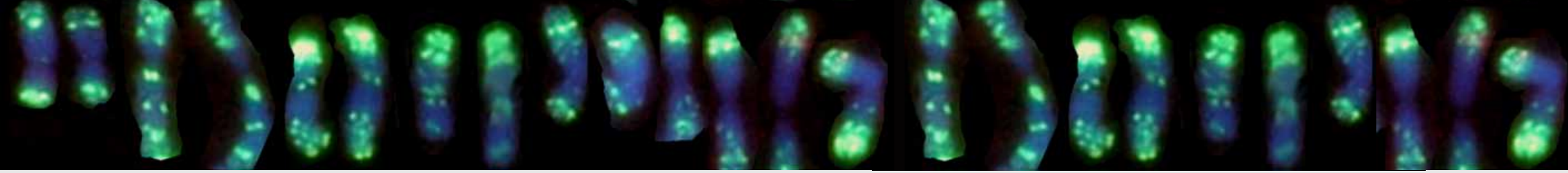
strangulata genotype are distributed along the Caspian Sea shore (direction 2)





(Aegilops tauschii)

An evolutionary model supported by molecular analyses



- 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 -

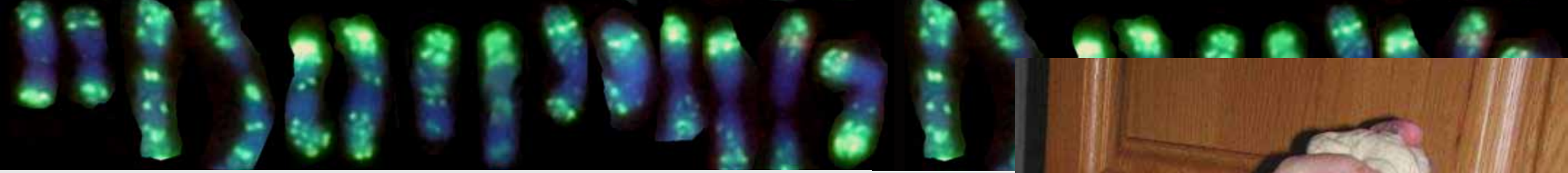
Aegilops

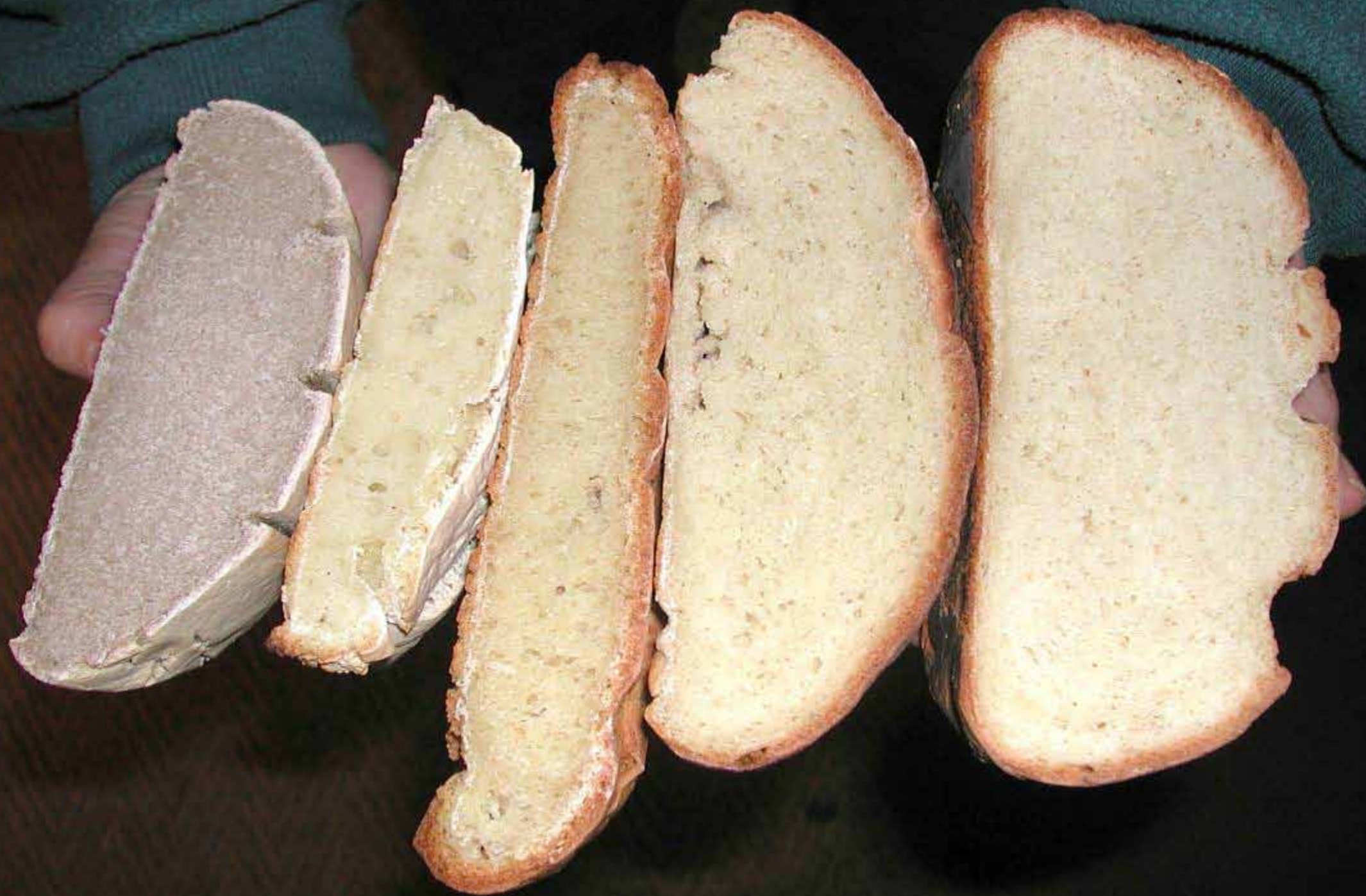
Triticum durum



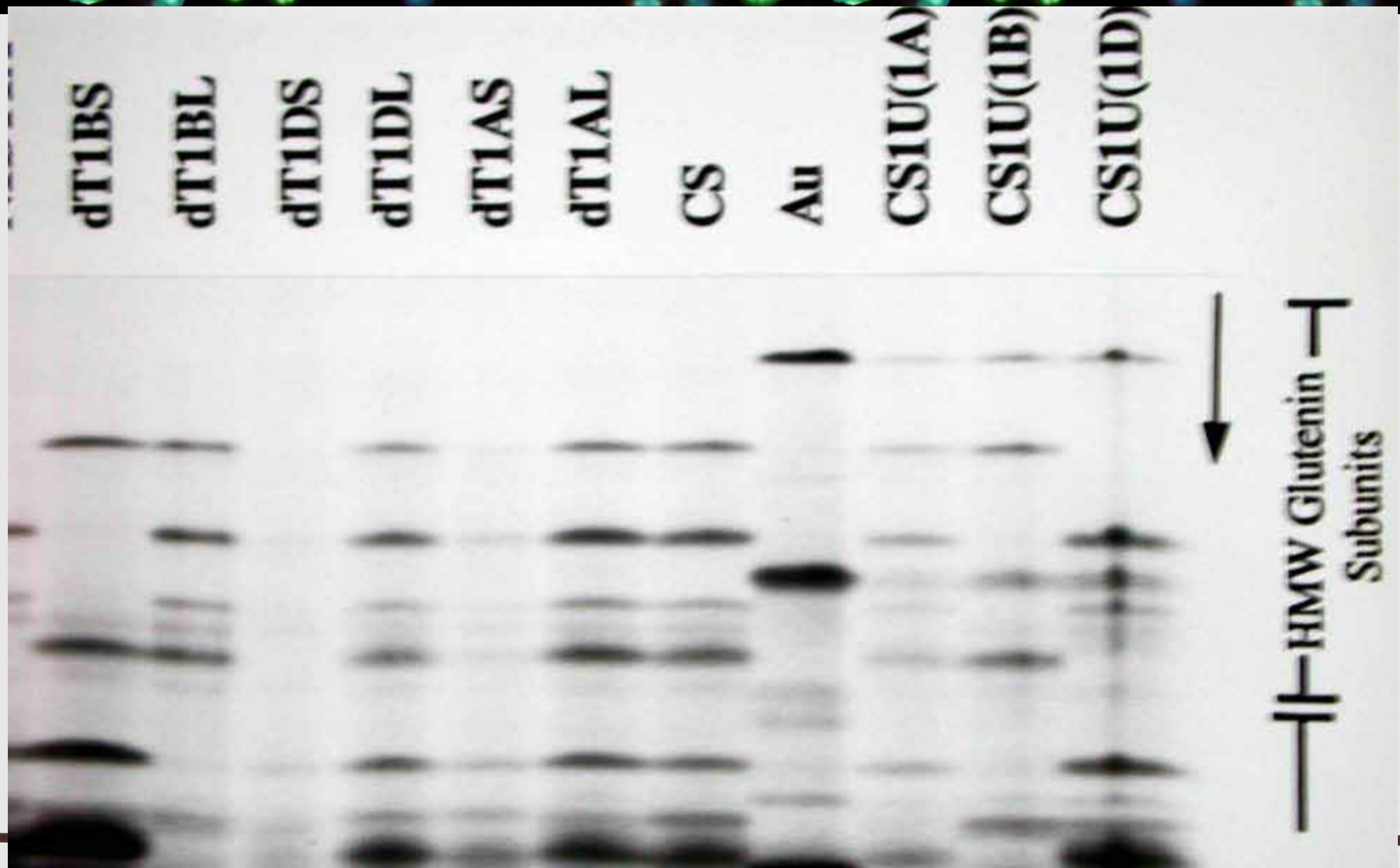
Ae. umbellulatta type ' *T. durum*

C0175 Espanhol 8914





Wheat proteins: High bands = good bread



Wheat - *Aegilops umbellulata*
substitution line A94

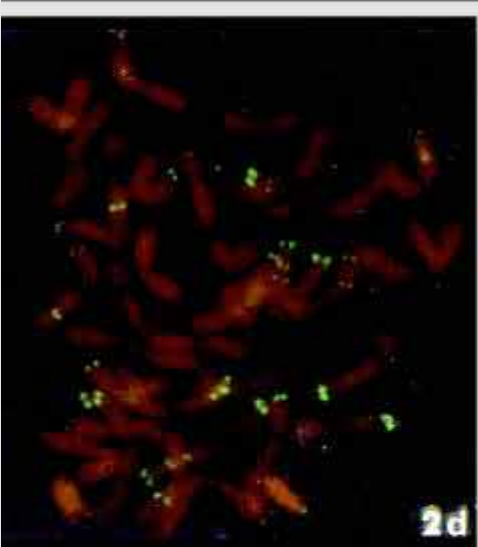
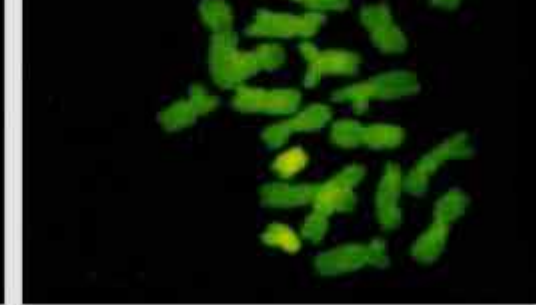
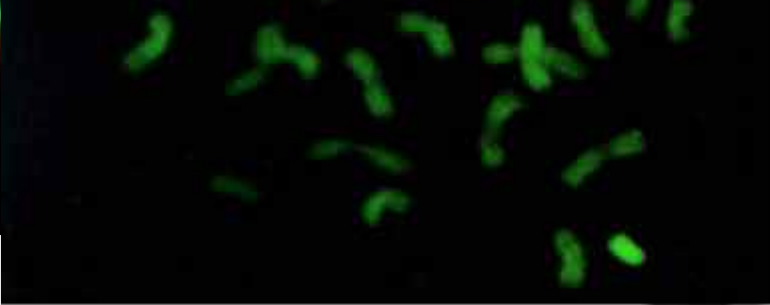
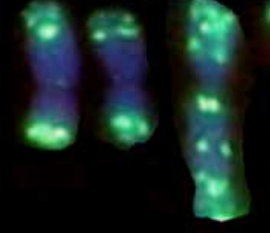
$2n=6x=42$

Genomic DNA from
Ae. umbellulata

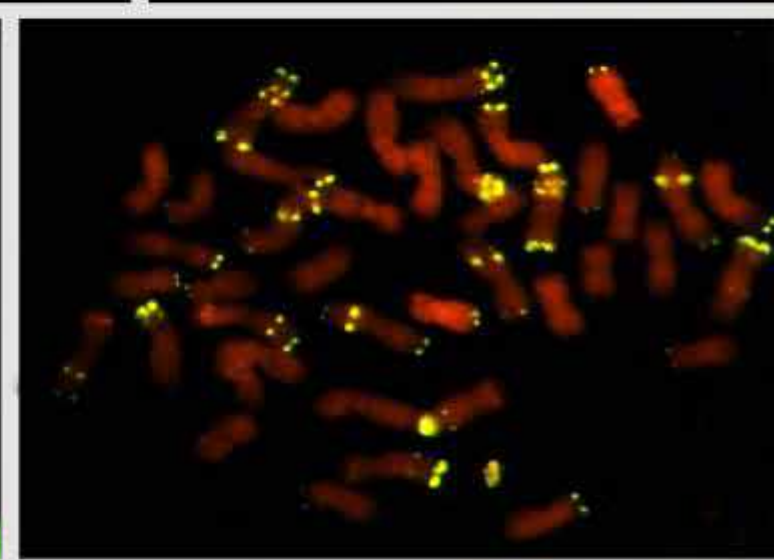
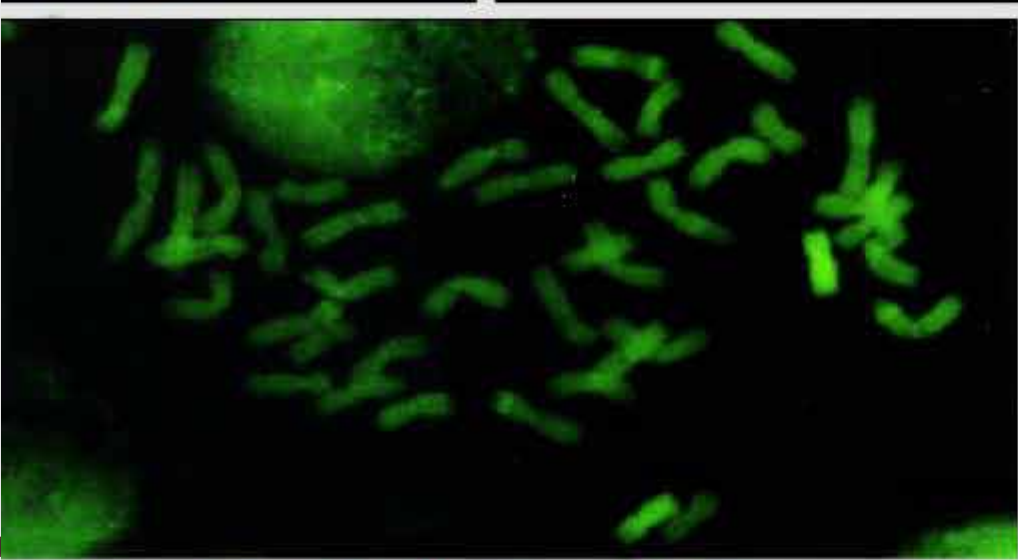
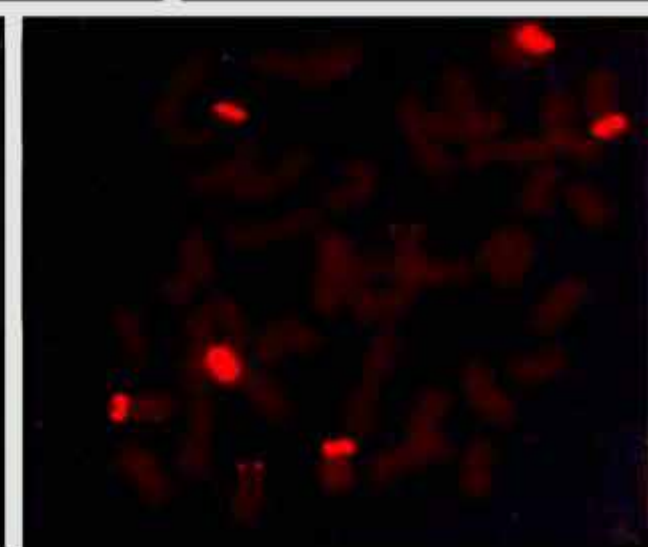
pSc119.2 repeat

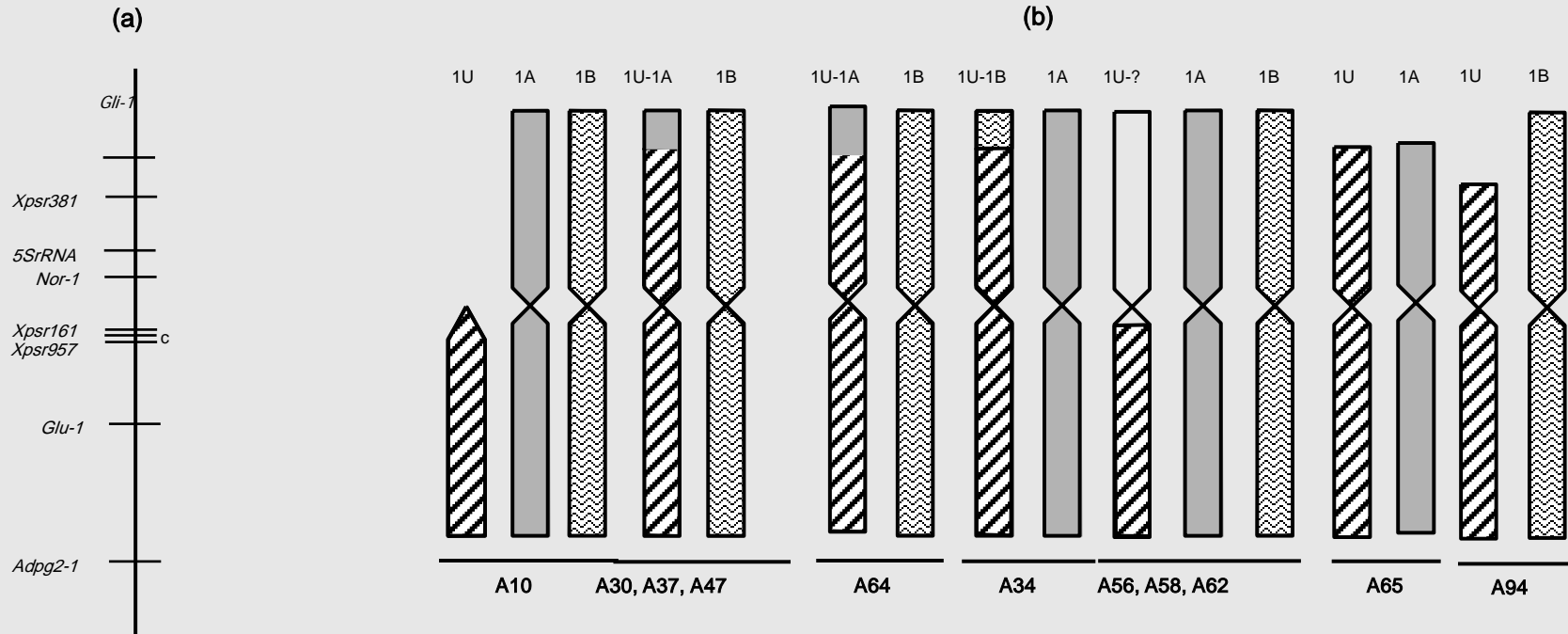
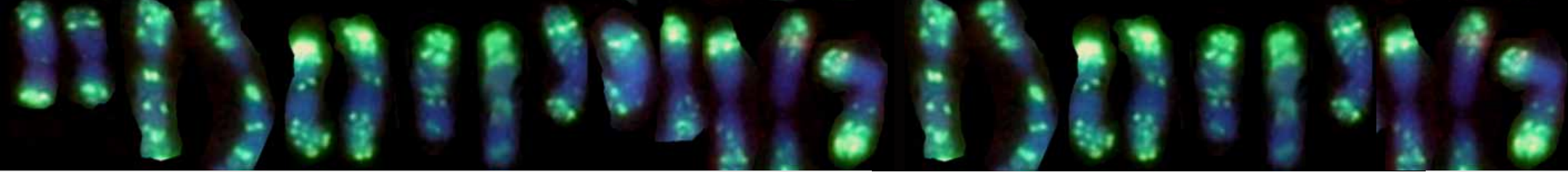


Xana Castilho



2d

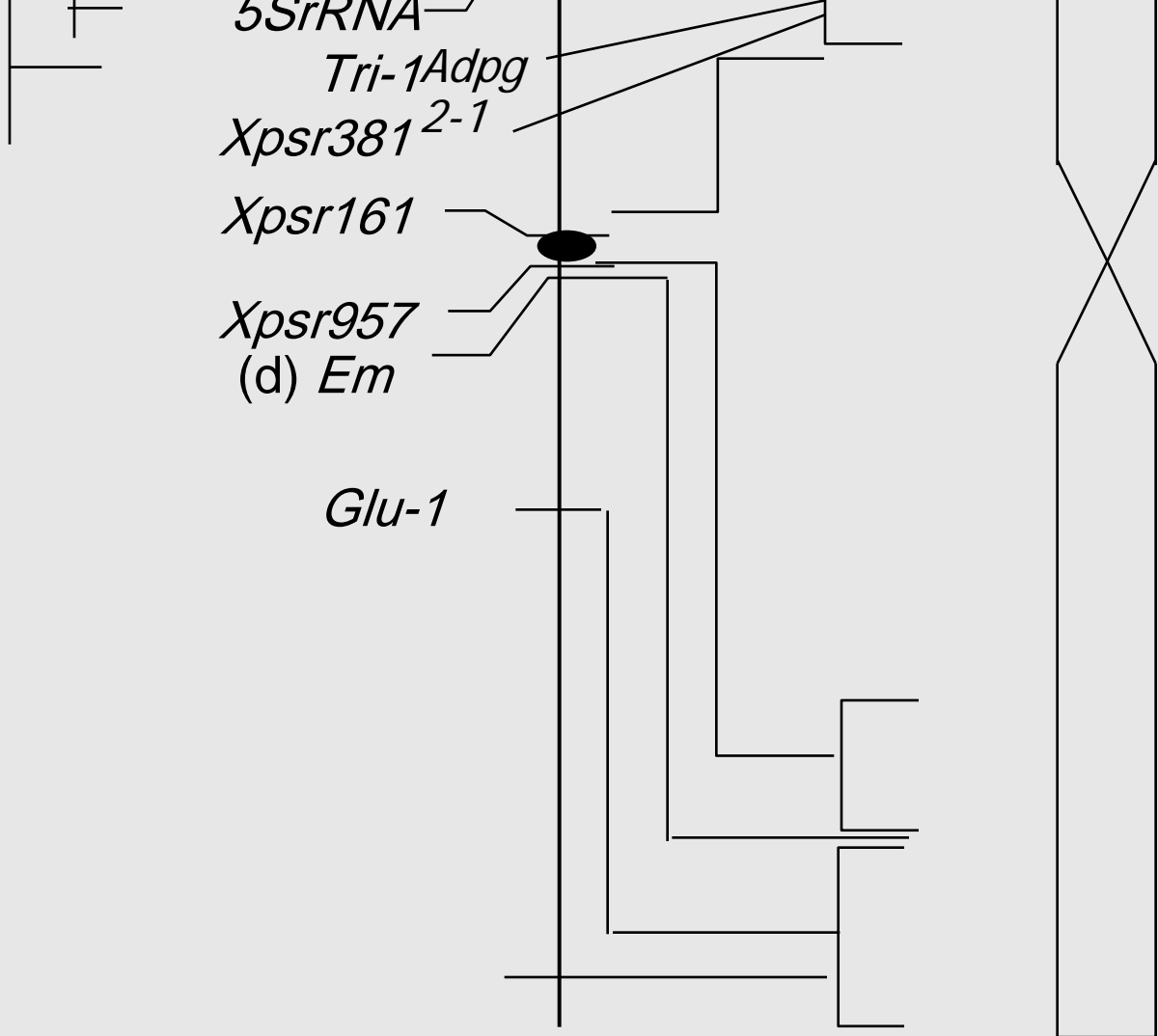
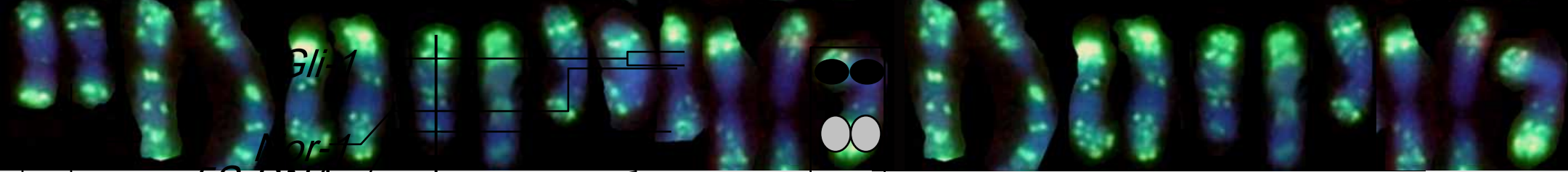




(a) Genetical map of the RFLP probes used. Genetical distances are approximate and based on Gale *et al.*, 1995 for the wheat 1B chromosome.


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

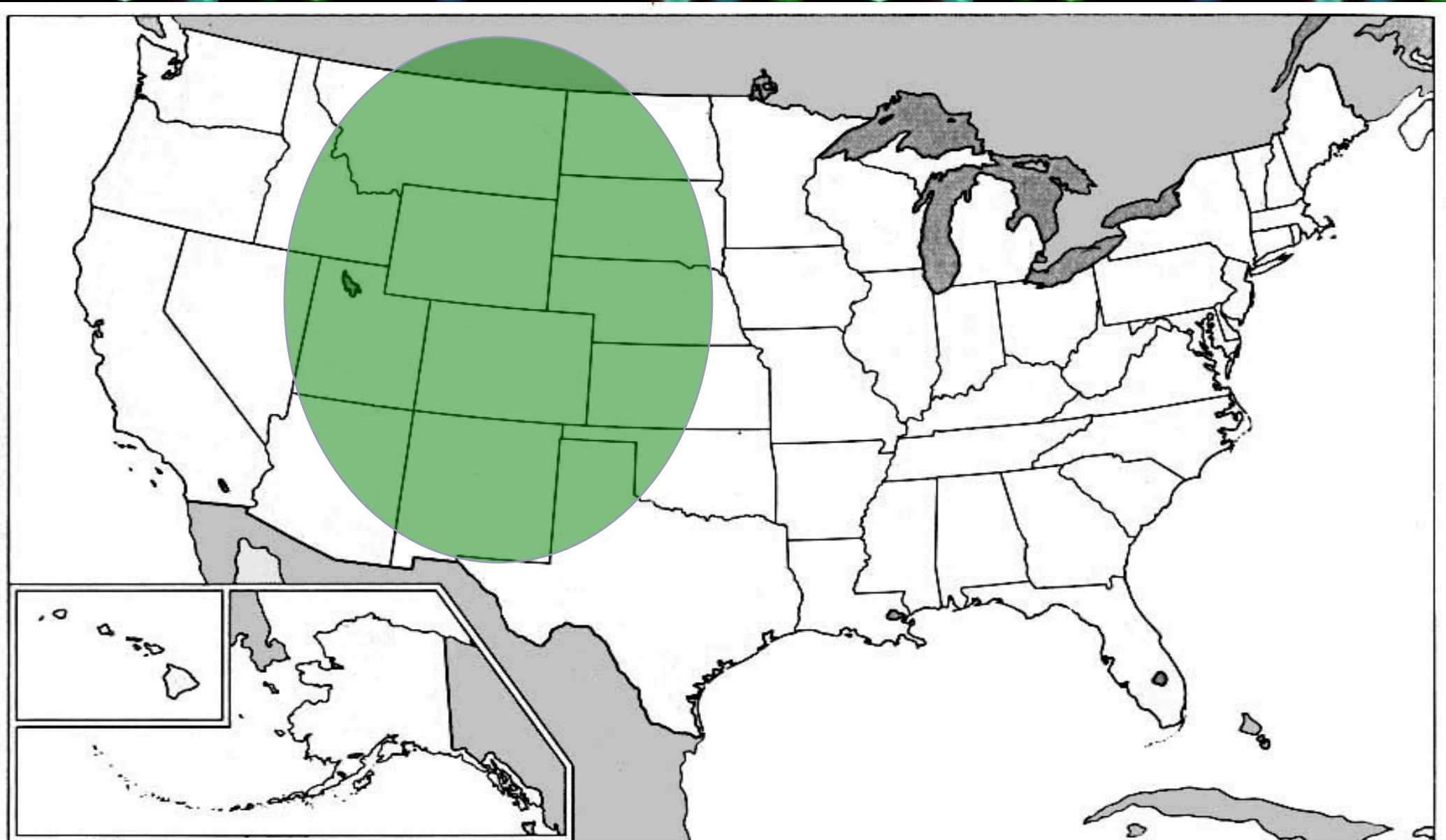


*Thinopyron
bessarabicum*
disomic addition
to wheat ($2n=6x+2=44$)

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 the wild grass are stained green. The chromosomes are arranged in a roughly circular pattern, typical of a karyotype preparation.

Wheat Streak Mosaic Virus in North America

Bob Graybosch, USDA

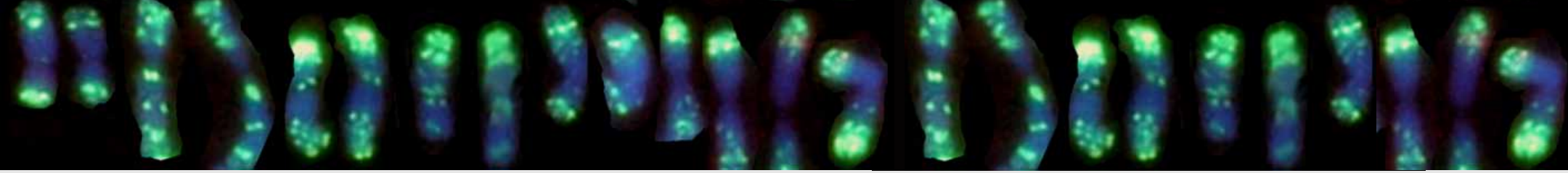




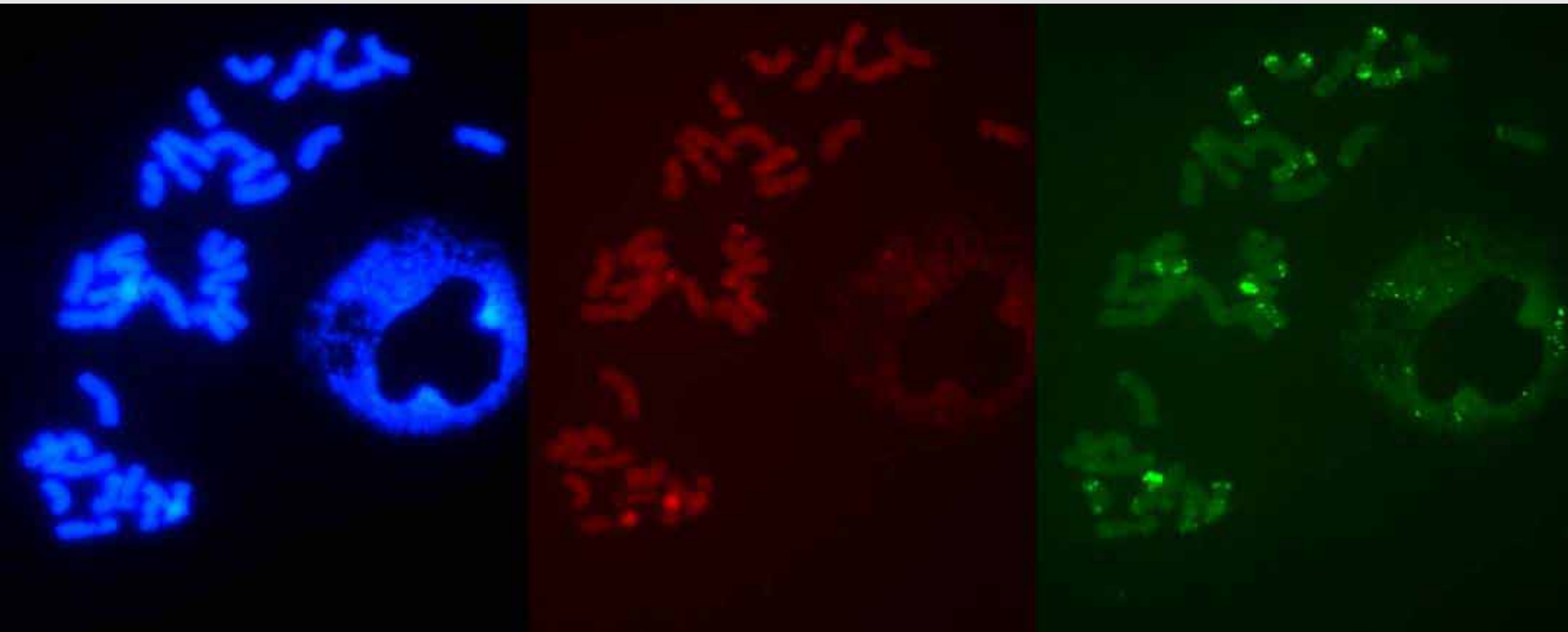
Wsm-1: only highly effective source of resistance to WSMV



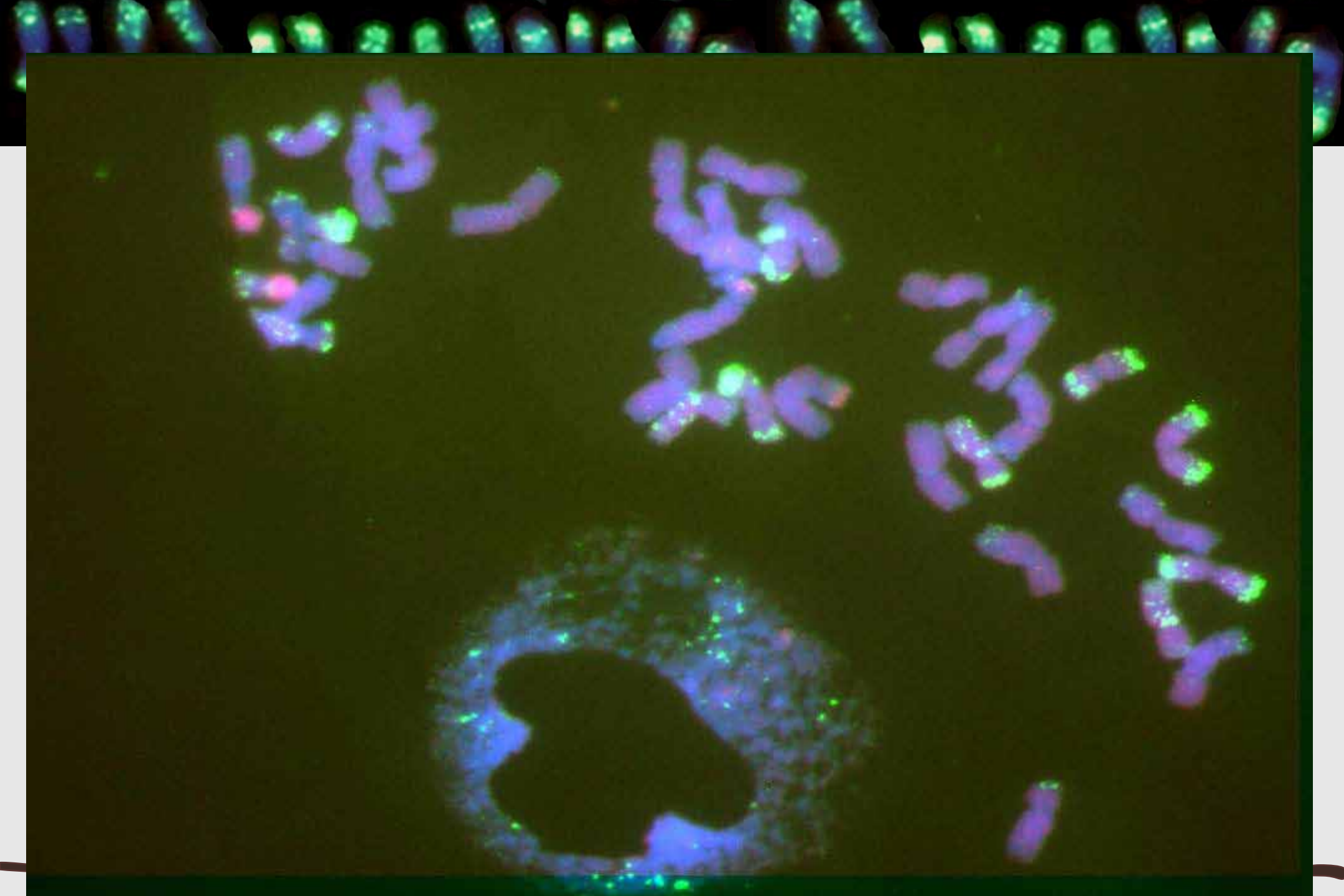
Intermediate wheat-grass, western Nebraska



IWG genomic DNA biotin



dpTa1 digoxigenin



Wsm 1 Resistance Gene

*Located on a small insert of chromatin from *Agropyron intermedium* (Horst.) Beauv. (= *Thinopyrum intermedium*) on wheat chromosome 4A or 4D.*

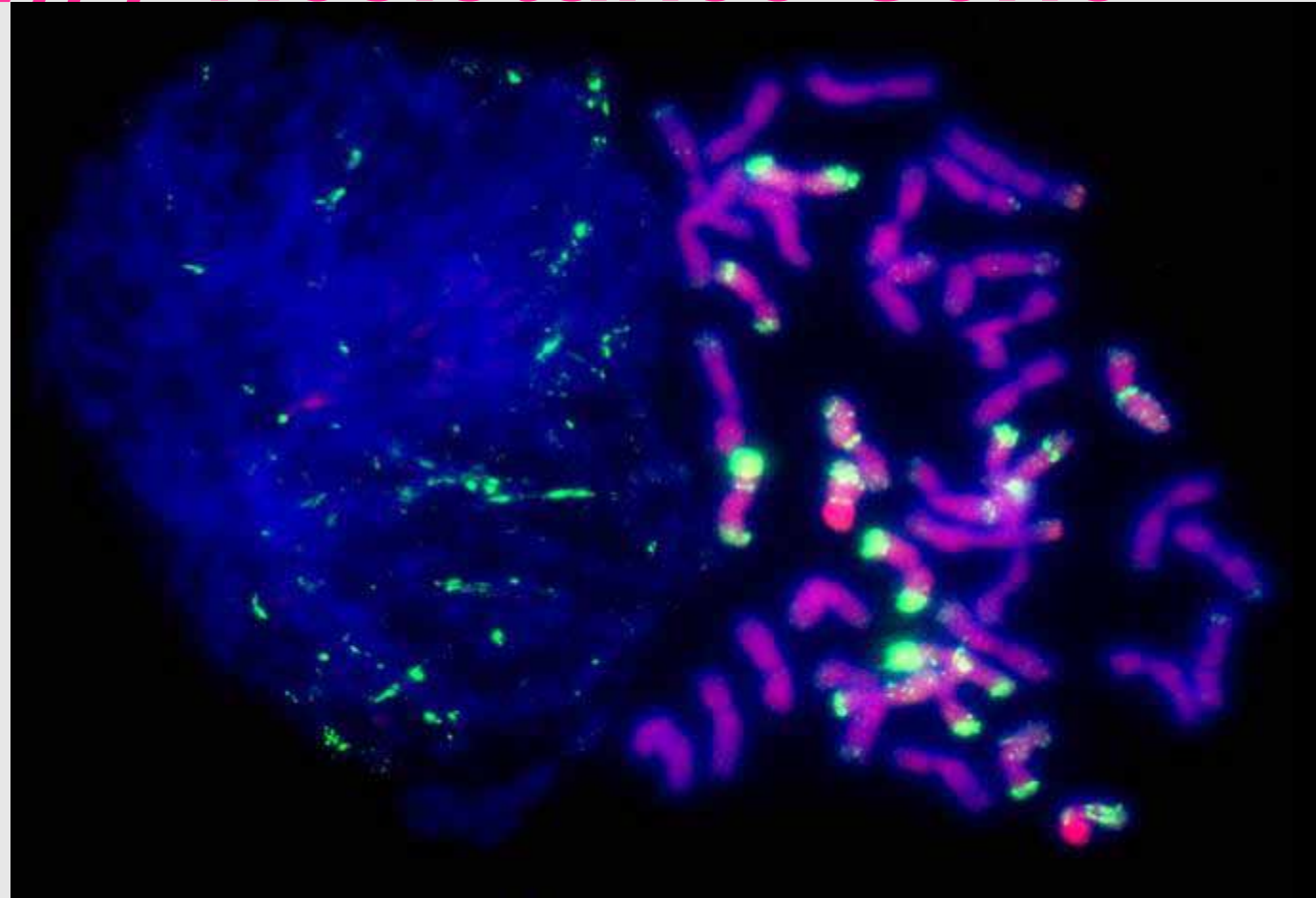
Goals at Leicester

- *What chromosome arm is present in our advanced breeding lines.*
- *Can we develop procedures to rapidly screen & characterize hundreds of germplasm lines?*

Probes

- *IWG genomic DNA – biotin*
- *dpTA1 (D-genome specific) digoxigenin*

Bob Graybosch, USDA



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*.

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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





Bos taurus taurus vs *Bos taurus indicus*:

$2n=60$, XY

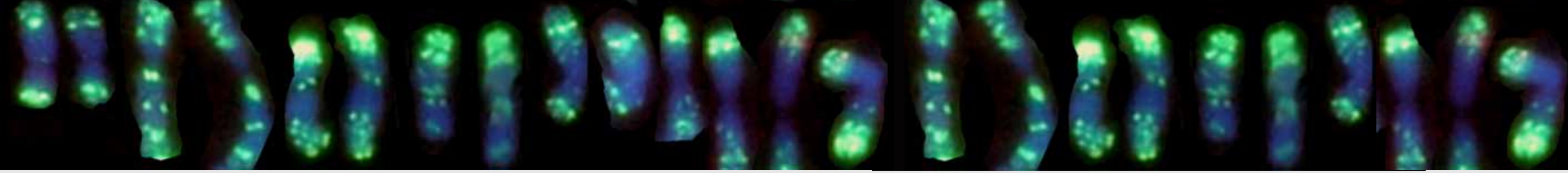
But: *B. taurus* submetacentric Y

B. indicus acrocentric Y

Bovidae

Cattle, sheep, goats, and antelopes

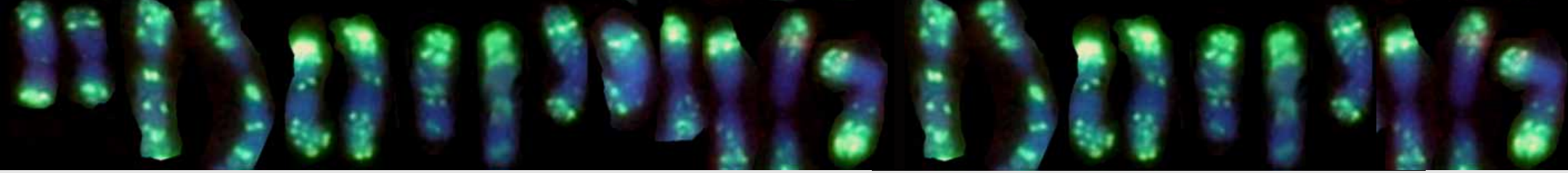




Bovidae – Family

☞ Mammals

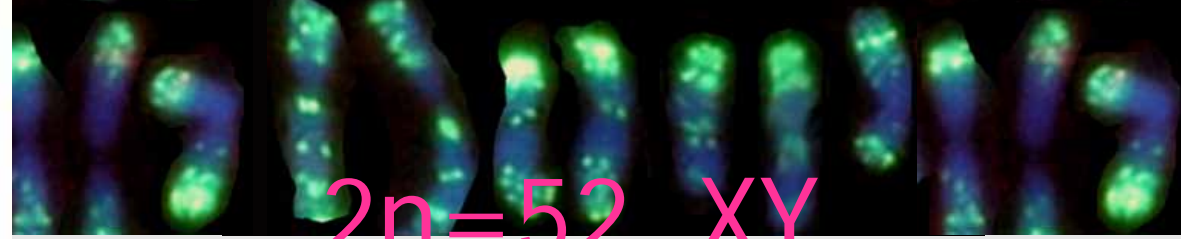
- Order Artiodactyla (=Even-toed ungulates)
- 3 groups: the Suiformes (pigs, peccaries, hippopotamuses), Tylopoda (camels, llamas) and Ruminantia (cattle, goats, sheep, deer, antelopes, giraffes)
- 9 families (13 tribes) including Bovidinae
 - Family Bovidae
 - c. 137 species
 - Last species (new genus)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



Bovidae family

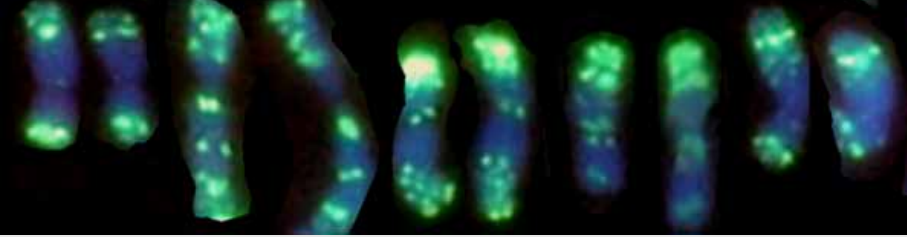
- $2n$ ranges from 30 to 60 in different species

- (Galagher & Womak 1992)

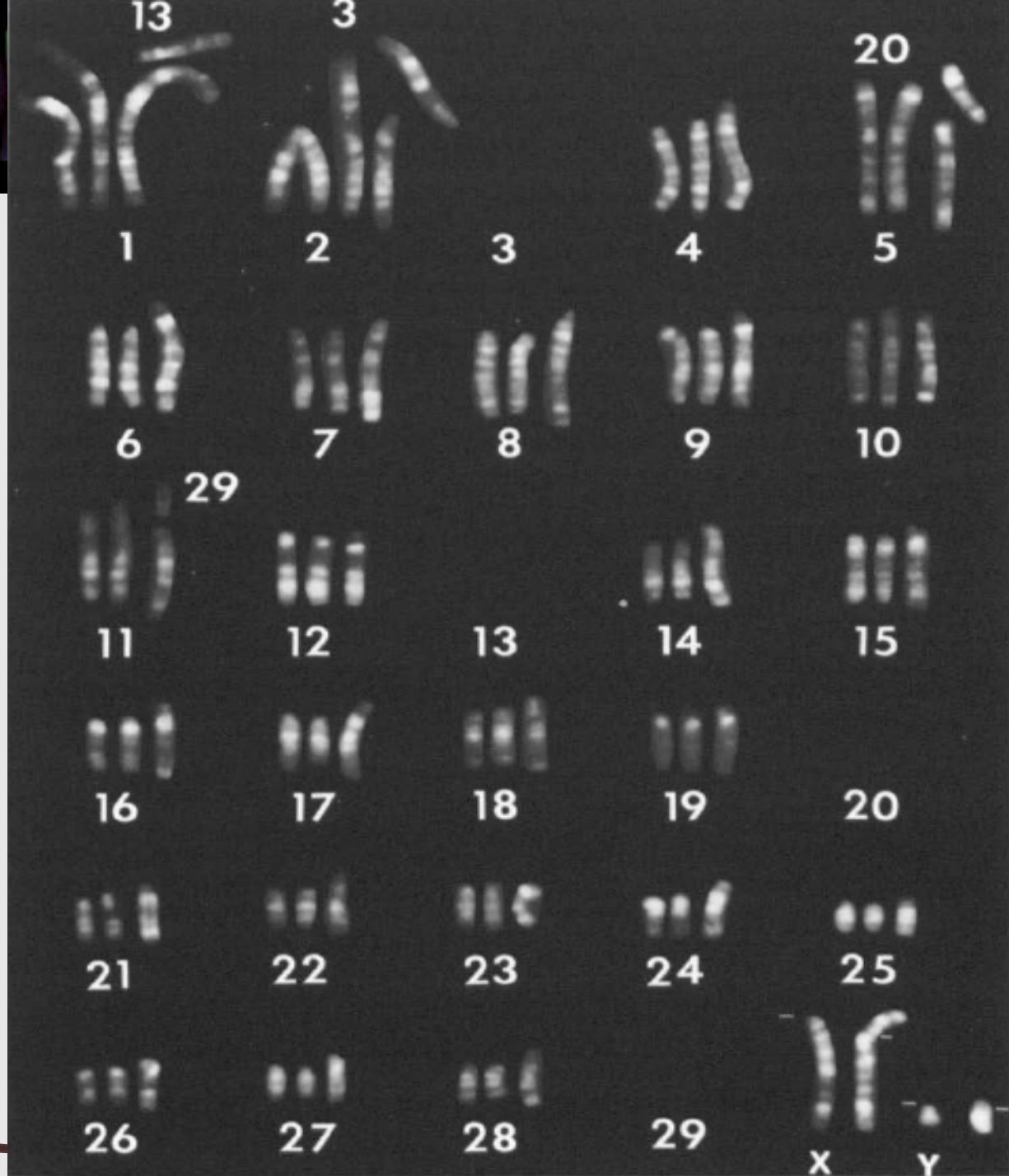


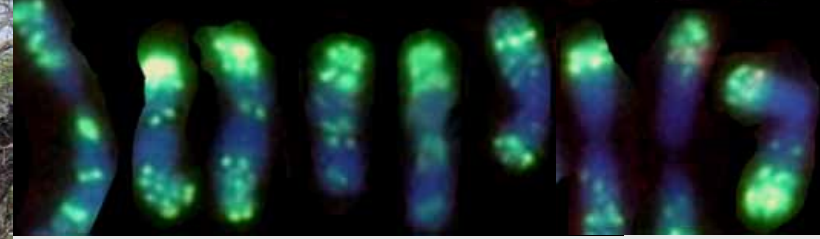
$2n=52, XY$
including 4
bi-armed
chromosomes = 58
autosomal
chromosome arms
+ X, Y

- *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





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

Tragelaphus strepsiceros or greater kudu

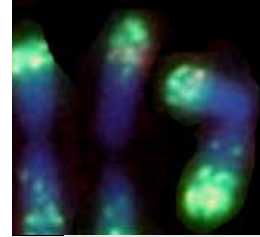
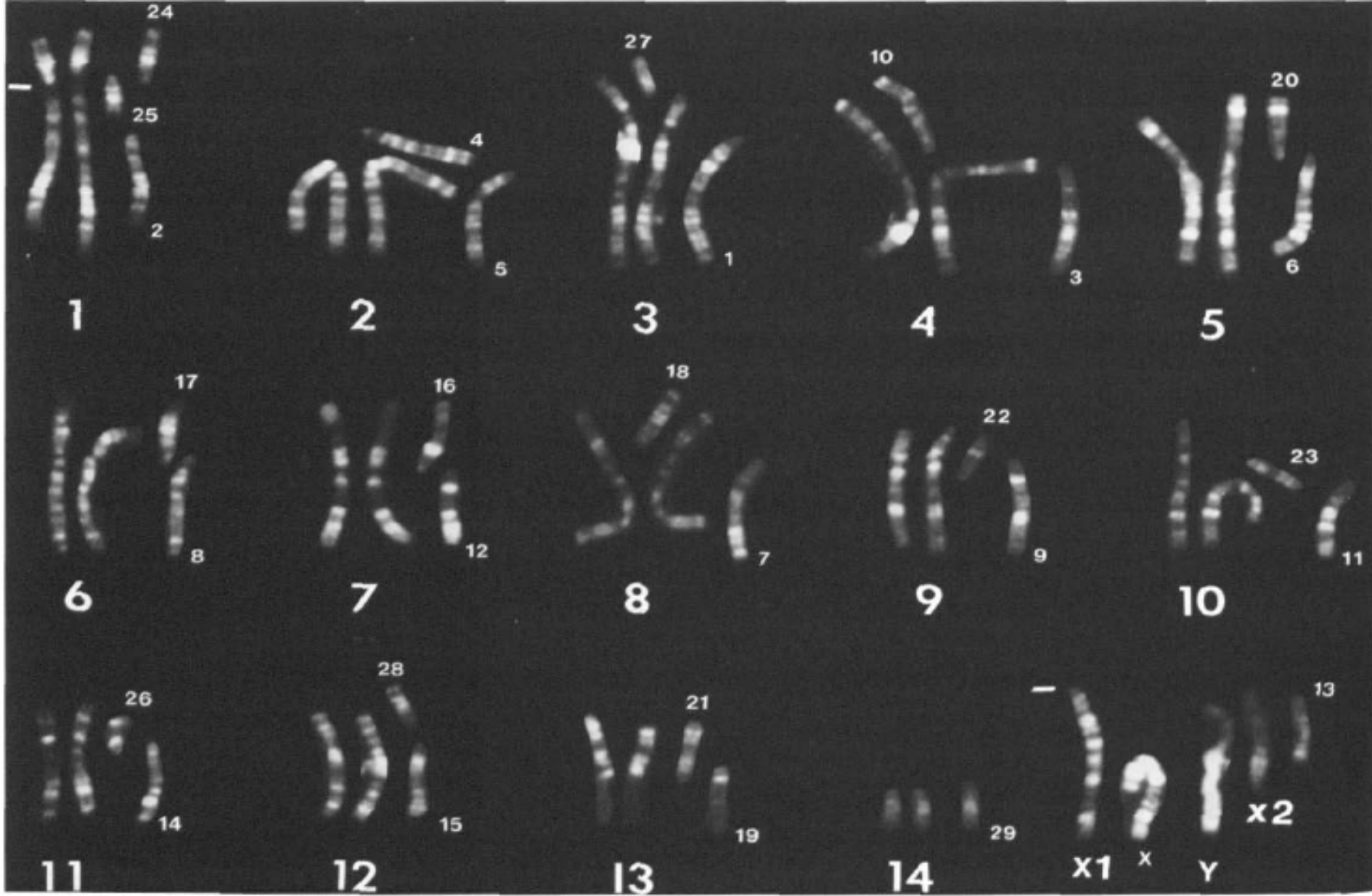
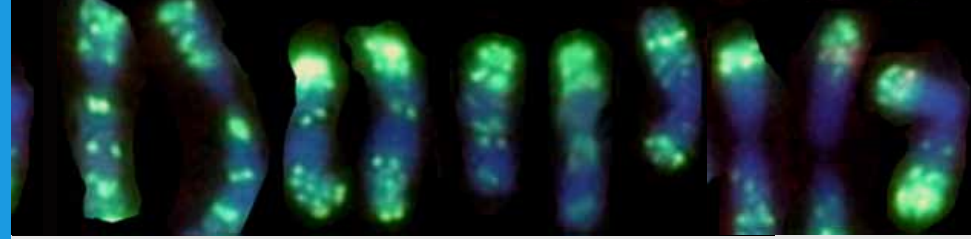
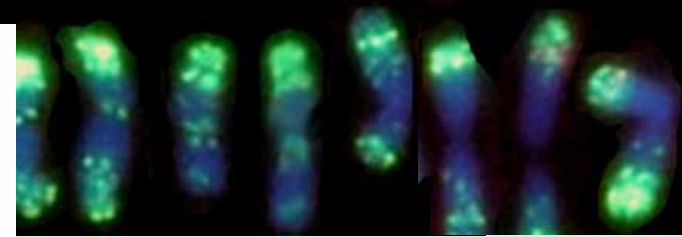


Figure 4. A male *Tragelaphus strepsicerus* (greater kudu) QFH-band karyotype ($2n = 31$) consisting of 26 bialleled autosomes, three acrocentric autosomes (one labeled as X2), an acrocentric X1, and a bialleled Y (the ancestral Y is fused to cattle equivalent autosome 13). The autosomal pairs are arranged 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.

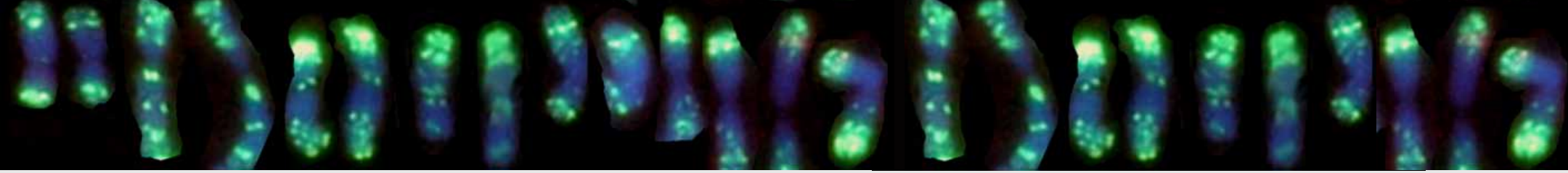


☛ Sheep *Ovis aries*

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



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



Bovidae Karyotype Evolution

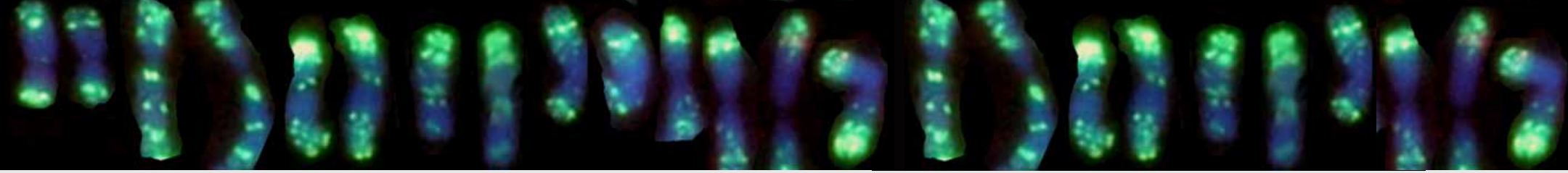
- ☞ $2n$ ranges from 30 to 60 in different species
- ☞ BUT:
- ☞ Almost all have 58 autosomal chromosome arms and two sex chromosomes
- ☞ (Galagher & Womak 1992)



Bovidae Karyotype Evolution

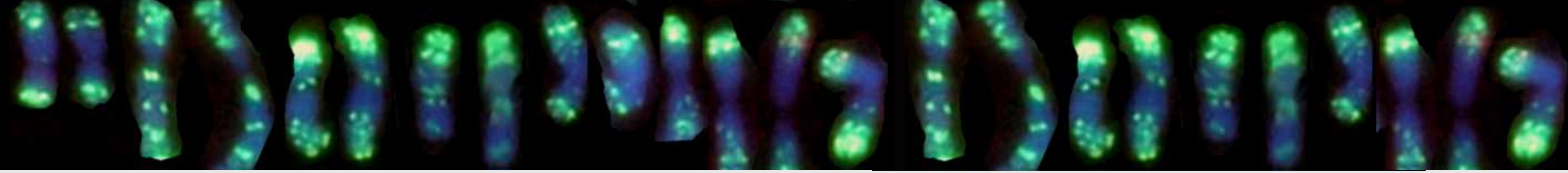
- ☞ Almost all have 58 autosomal chromosome arms and two sex chromosomes but $2n$ from 30 to 60
- ☞ Chromosome arm homologies extensive BUT homologous biarmed chromosomes are rare
- ☞ Reproductive isolation (and speciation) may have followed centric fusion

- ☞ (Galagher & Womak 1992)



Do we see chromosome fusion now?

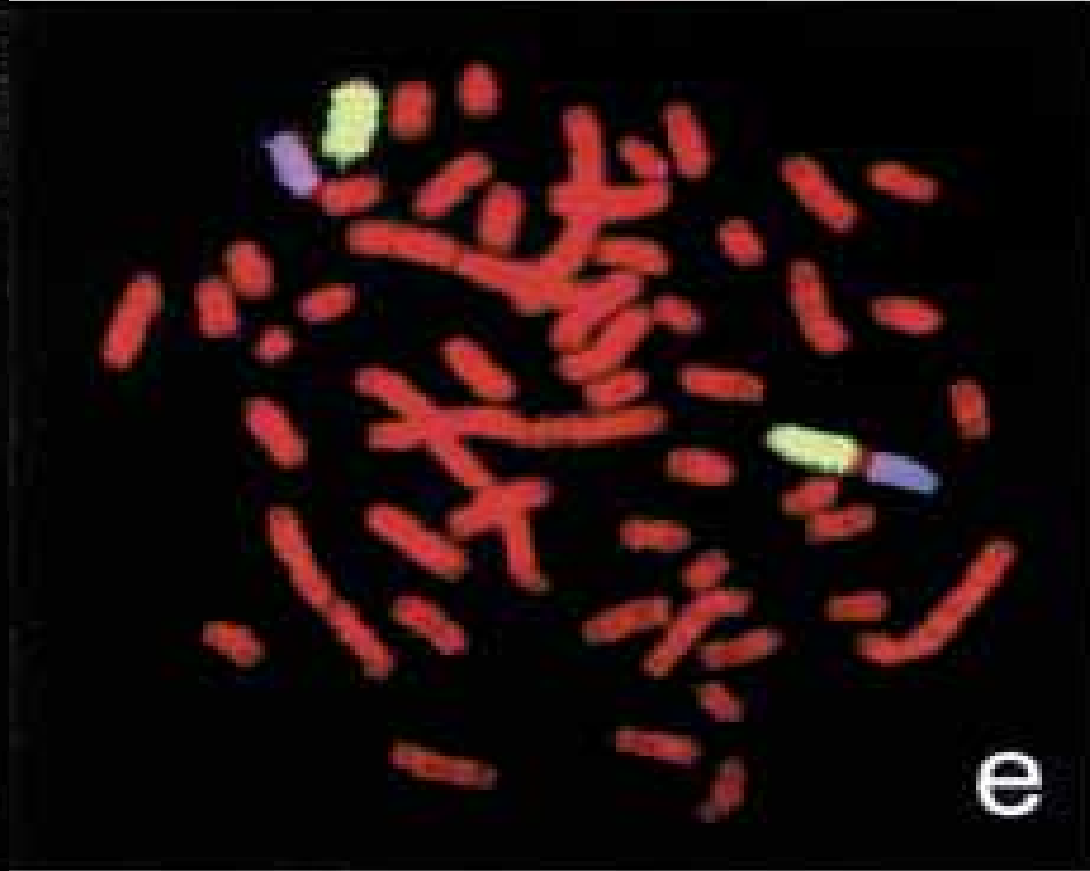
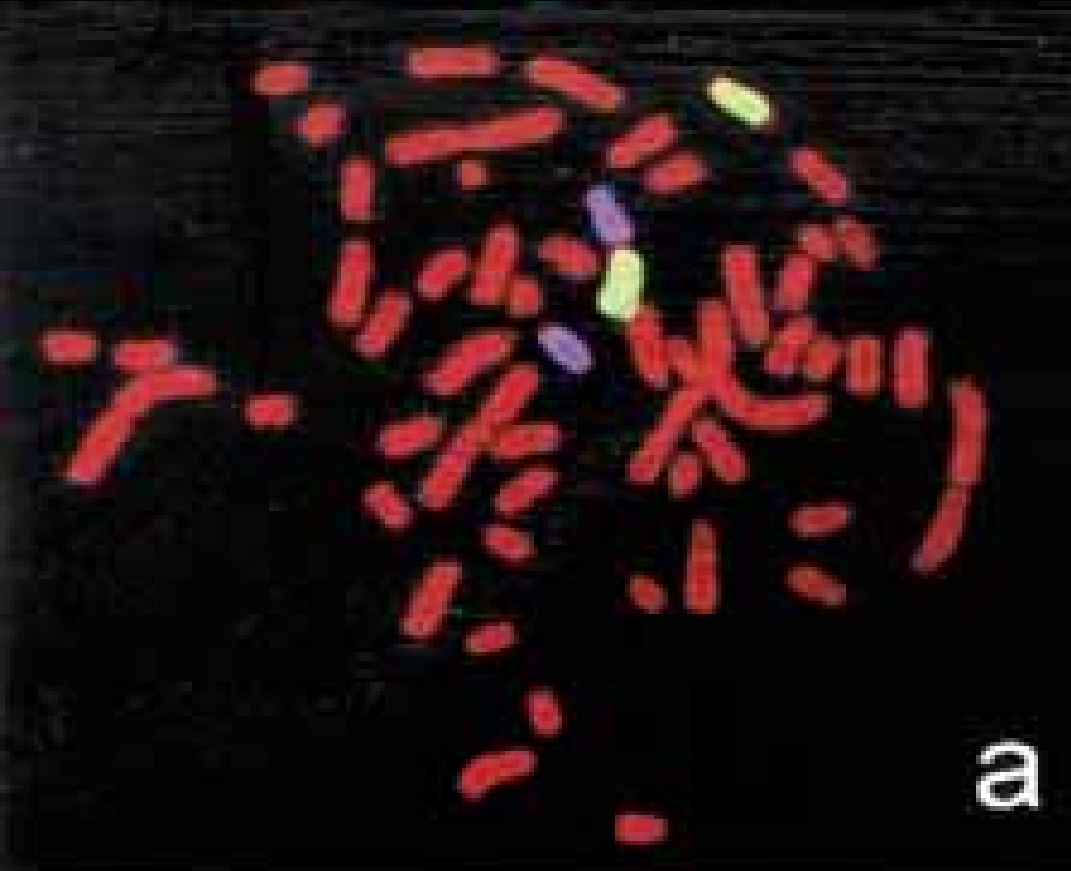
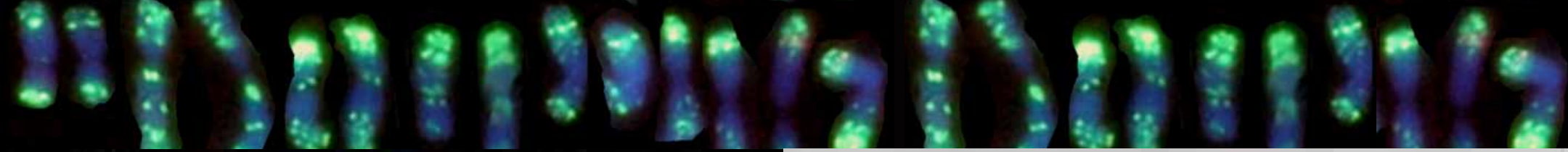


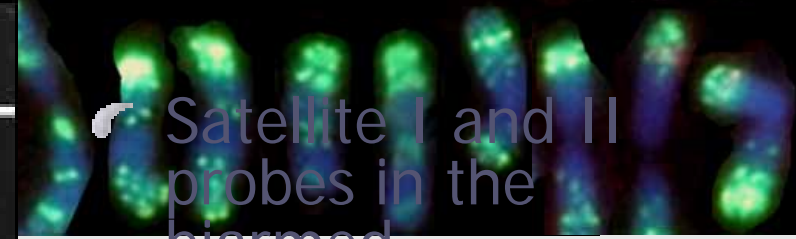
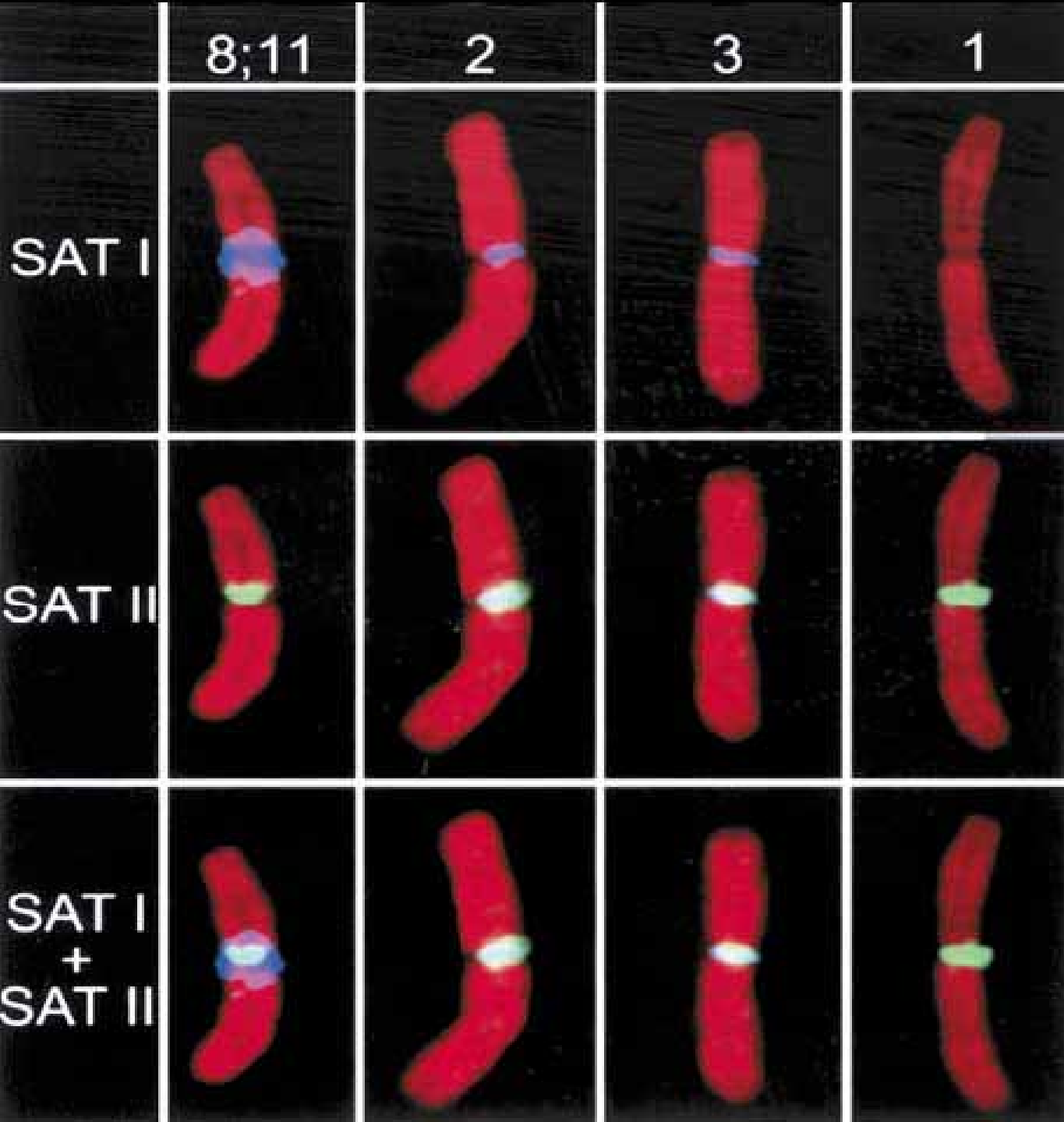


Do we see

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



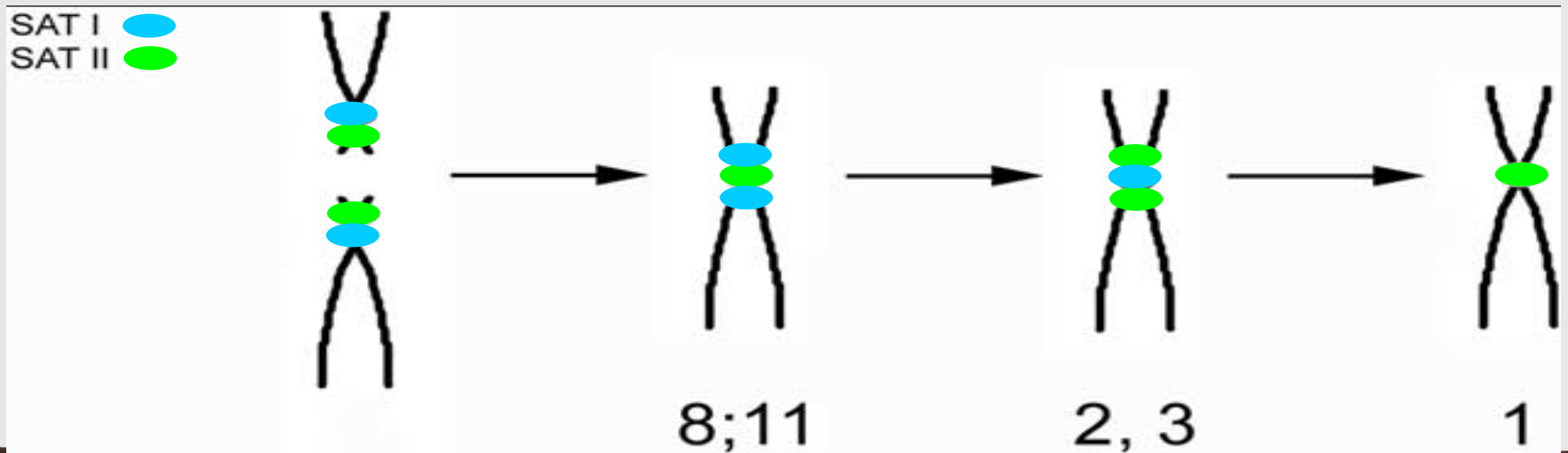




• Satellite I and II probes in the bivalents chromosomes of the sheep with $2n = 53, XY$.

• 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.



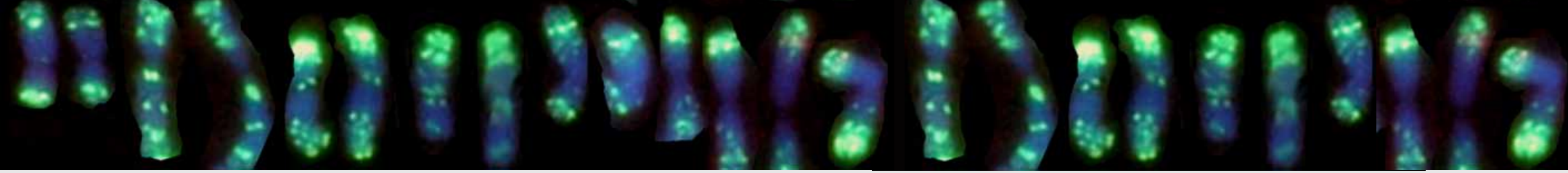
How many chromosomes?

☞ Is the number constant in a species?

☞ Cattle $2n=60$

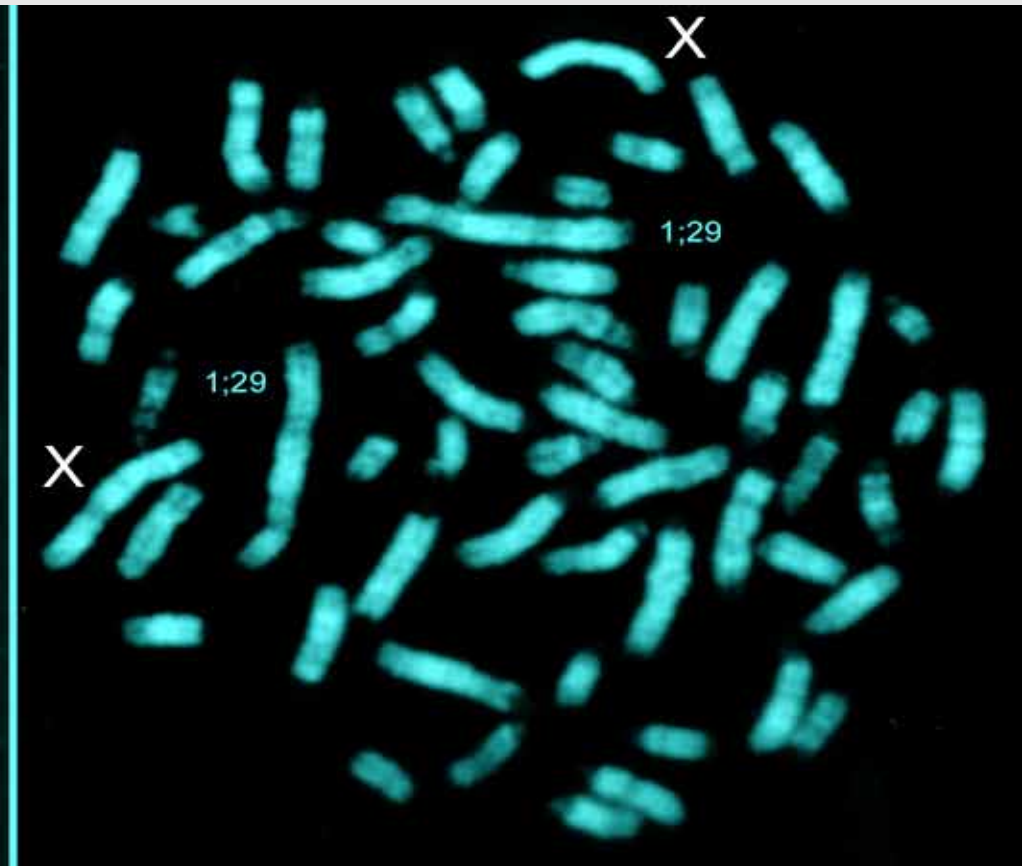
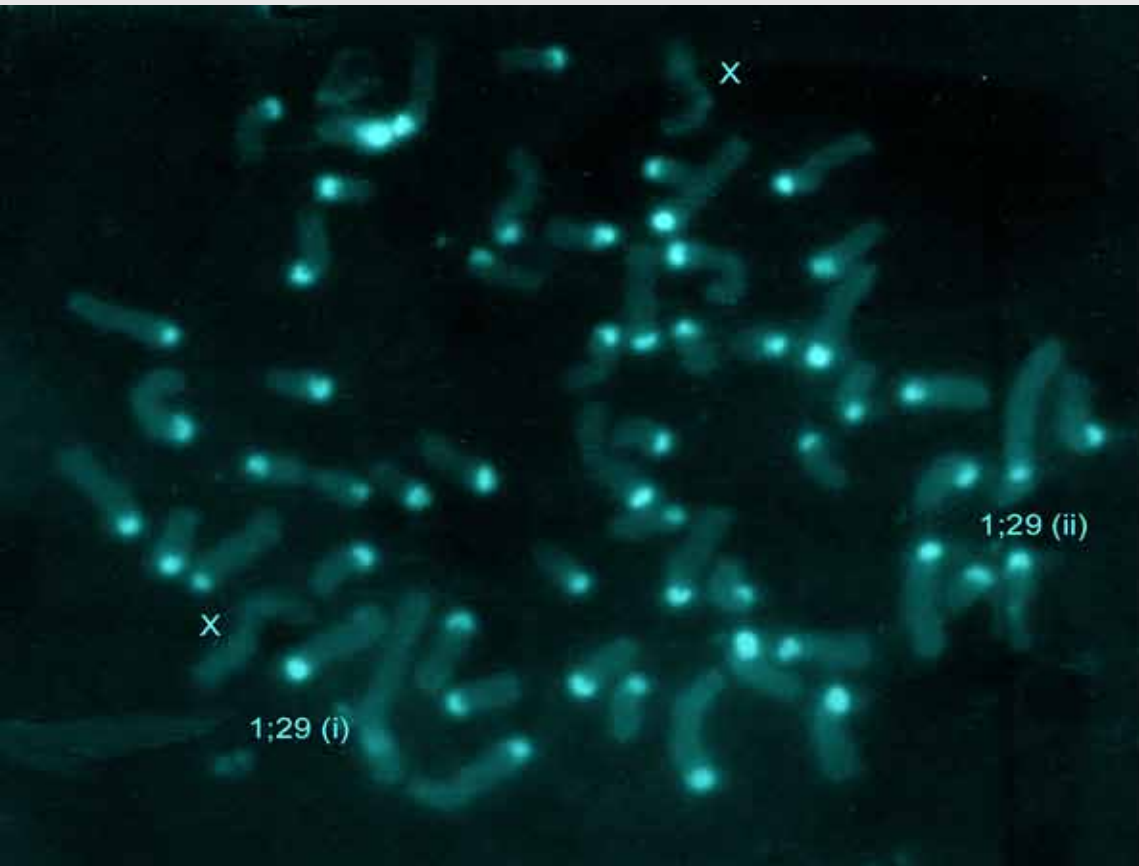
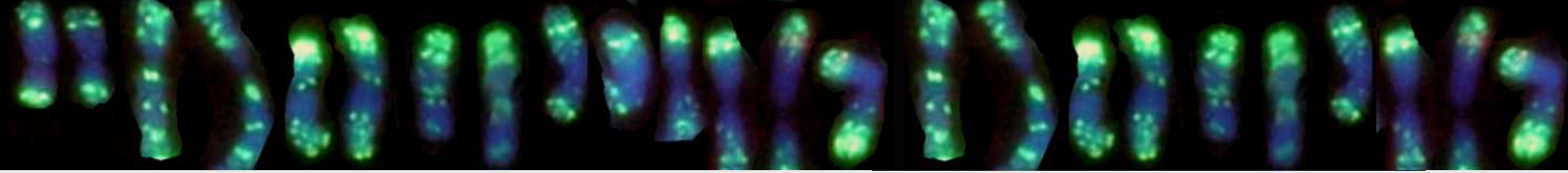
● but some individuals have $2n=58$
or $2n=59$ because two
chromosomes fuse

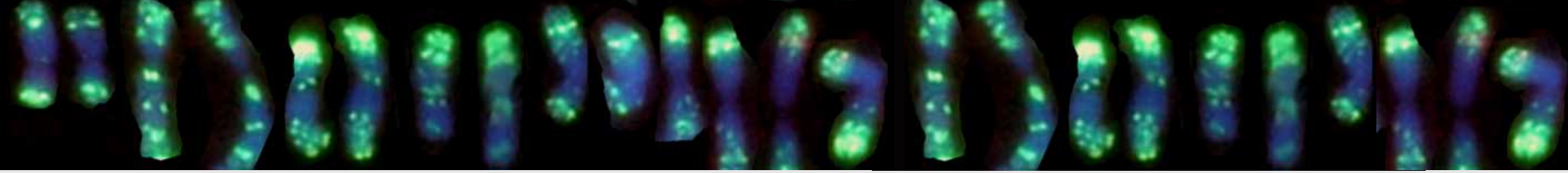
☞ Chromosomal evolution is happening now



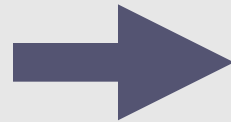
The 1;29 fusion in cattle

- 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

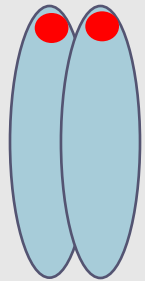




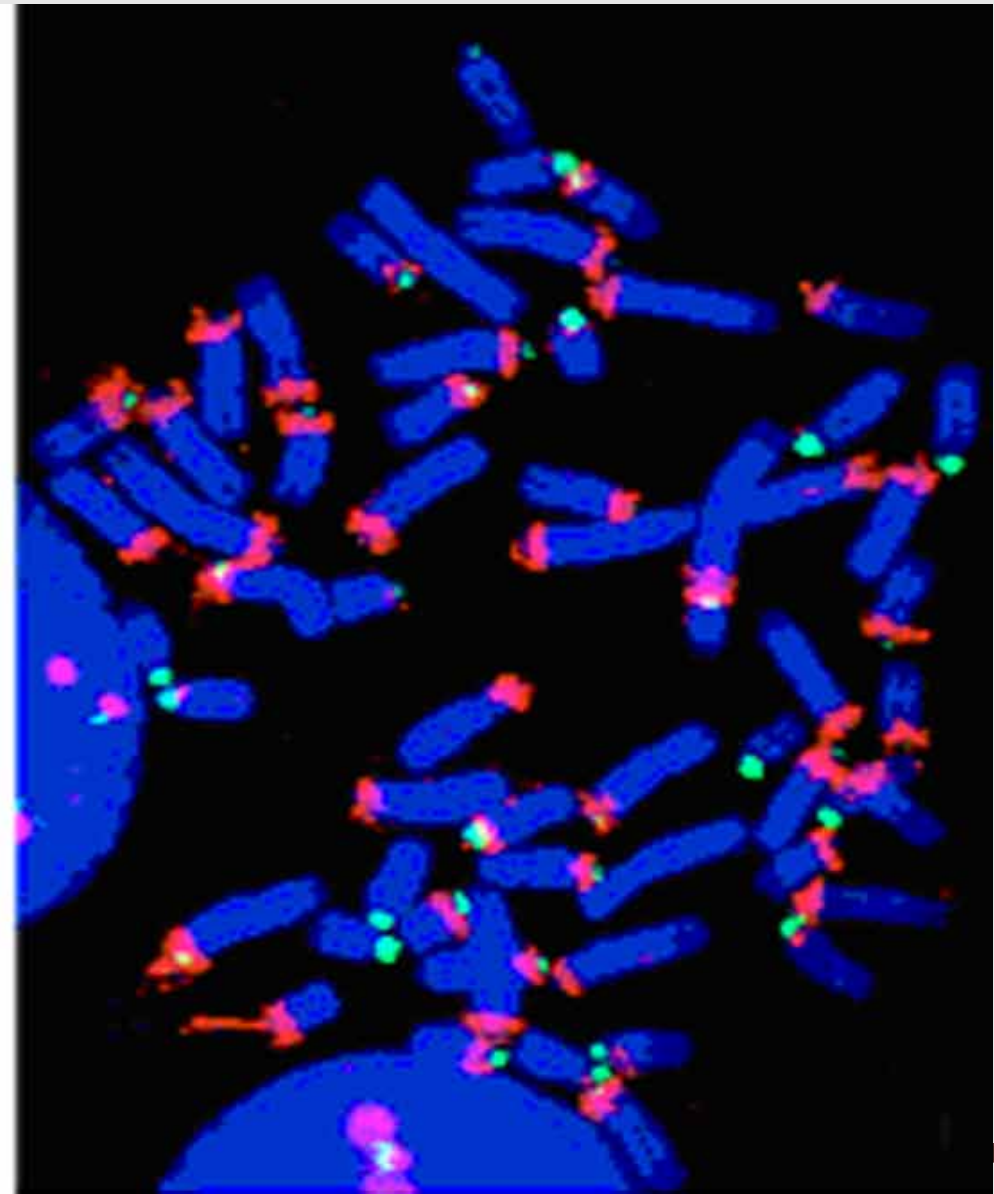
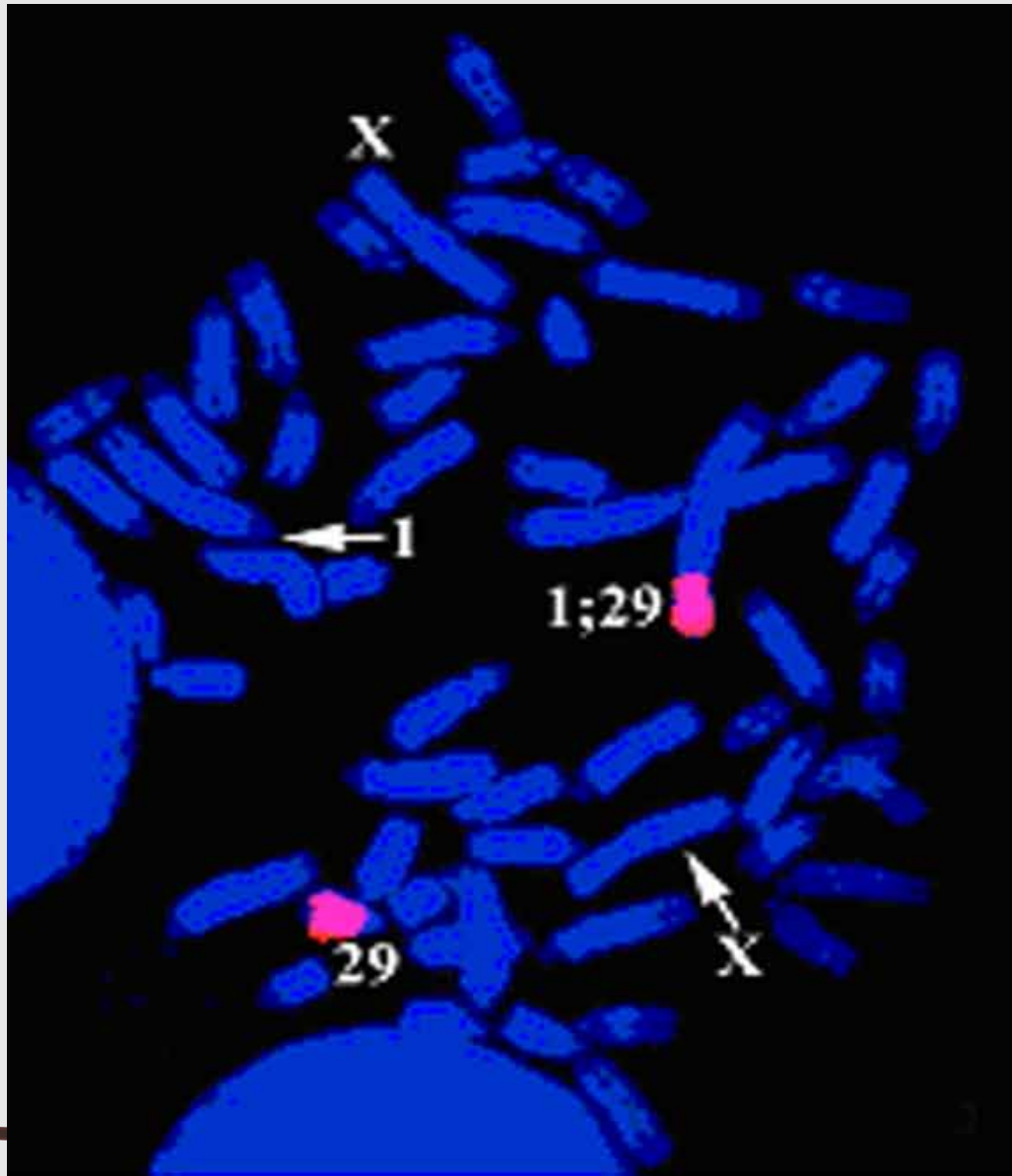
Robertsonian Fusion

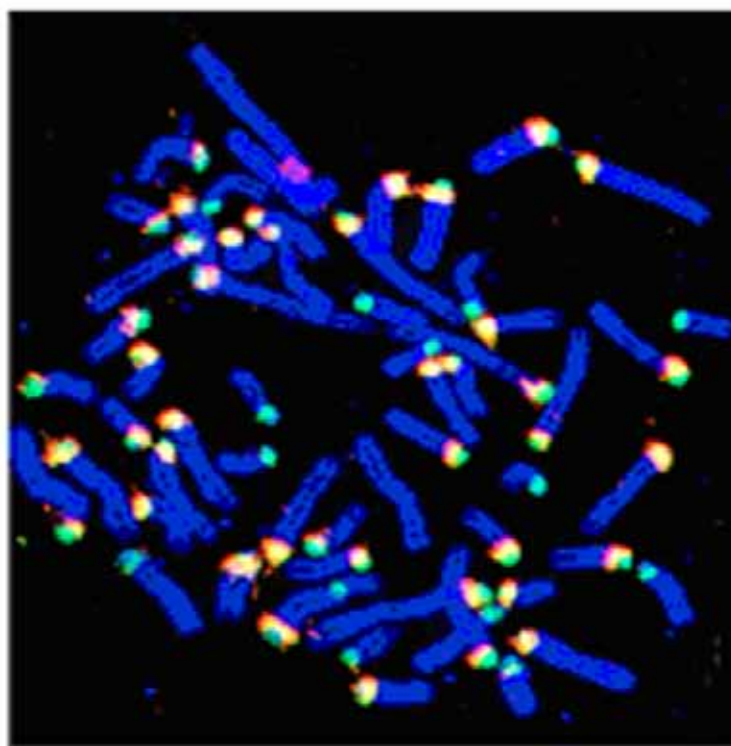
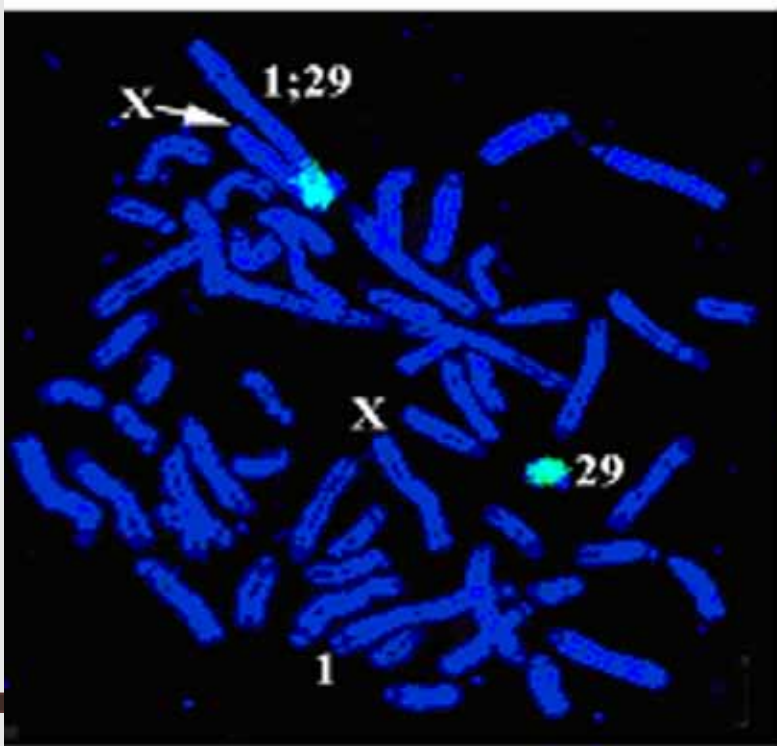
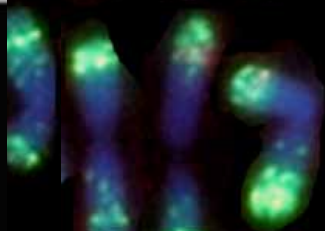
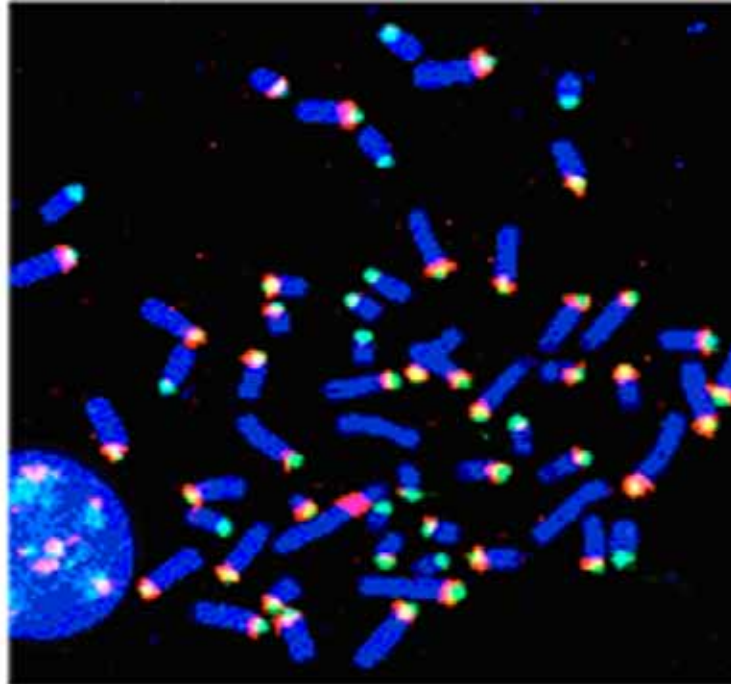
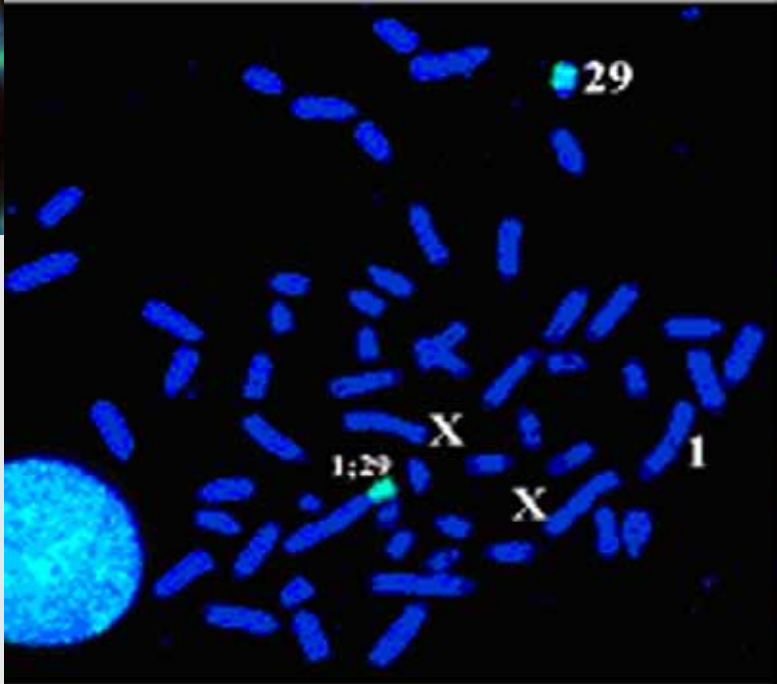
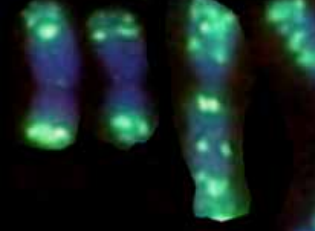


(+ ●● ?)



Bovine alpha-satellites and chromosome evolution

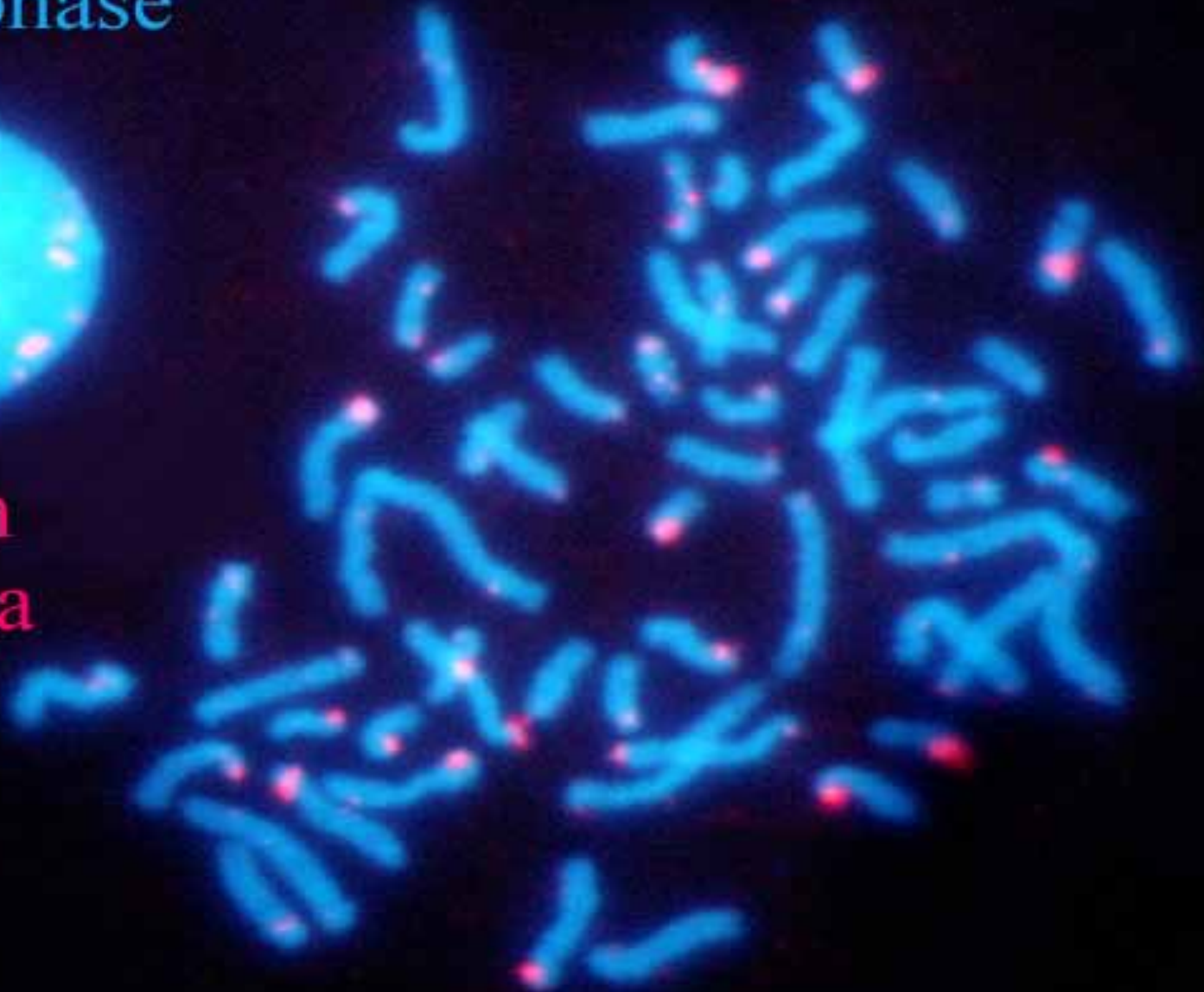




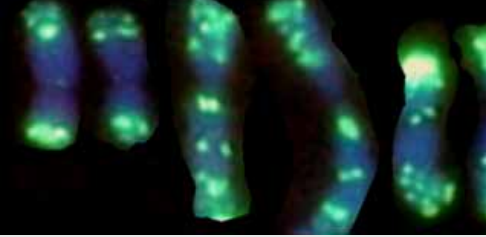
Goat Metaphase



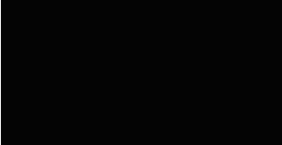
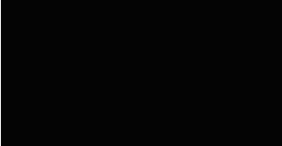
Probed with
Sheep Alpha
Satellite



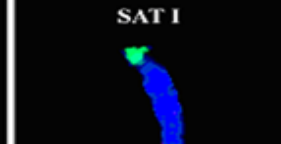
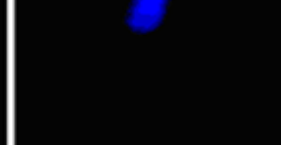
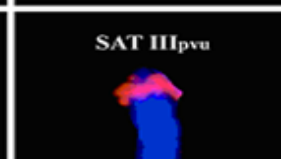
Raquel Chaves
& HH 1998



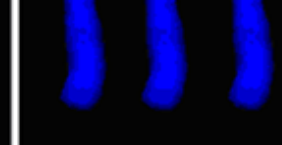
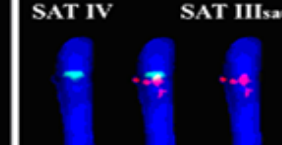
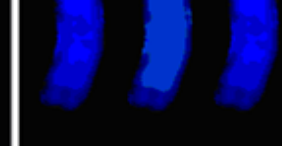
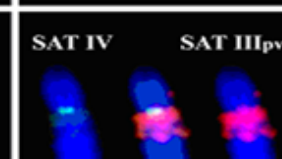
29



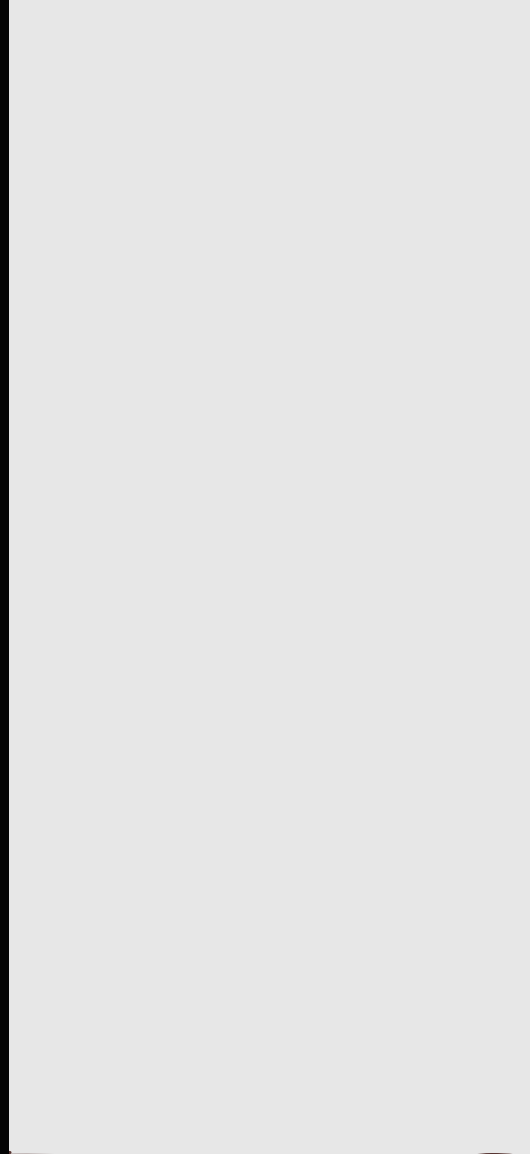
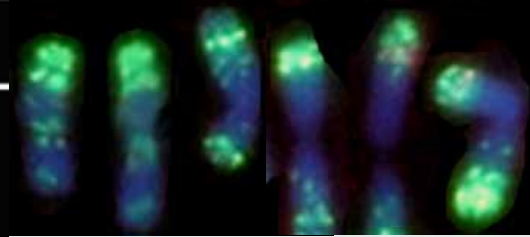
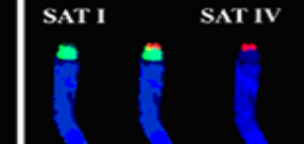
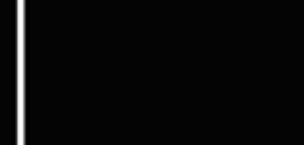
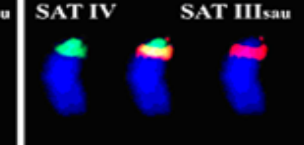
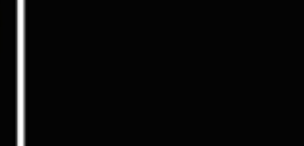
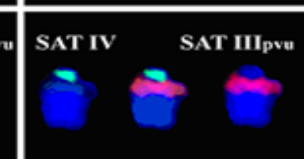
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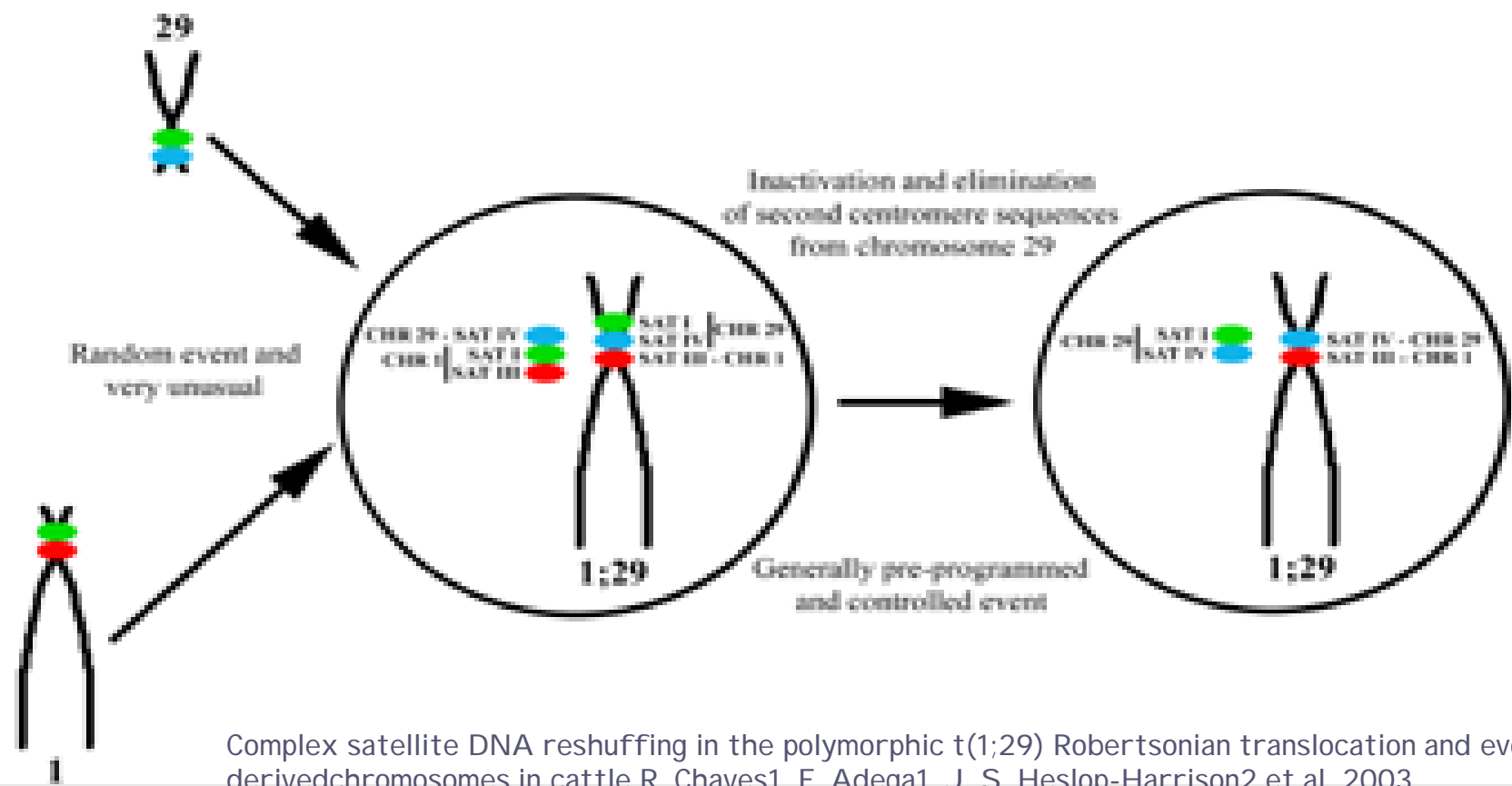
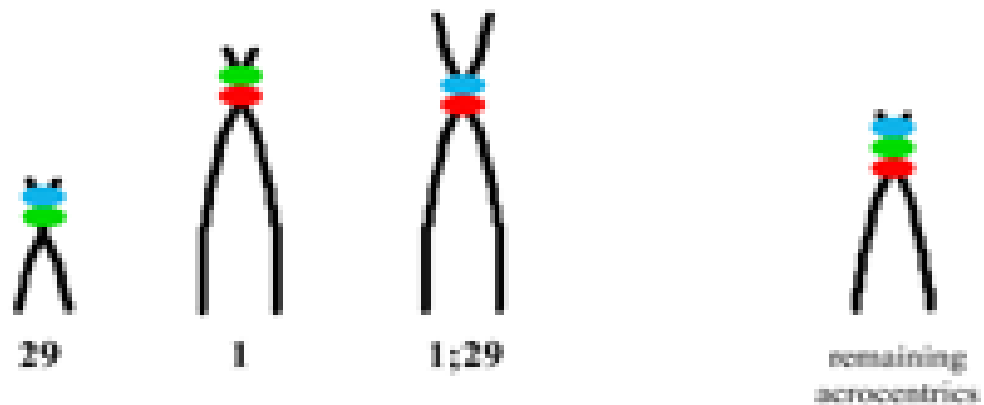
1;29



remaining acrocentrics



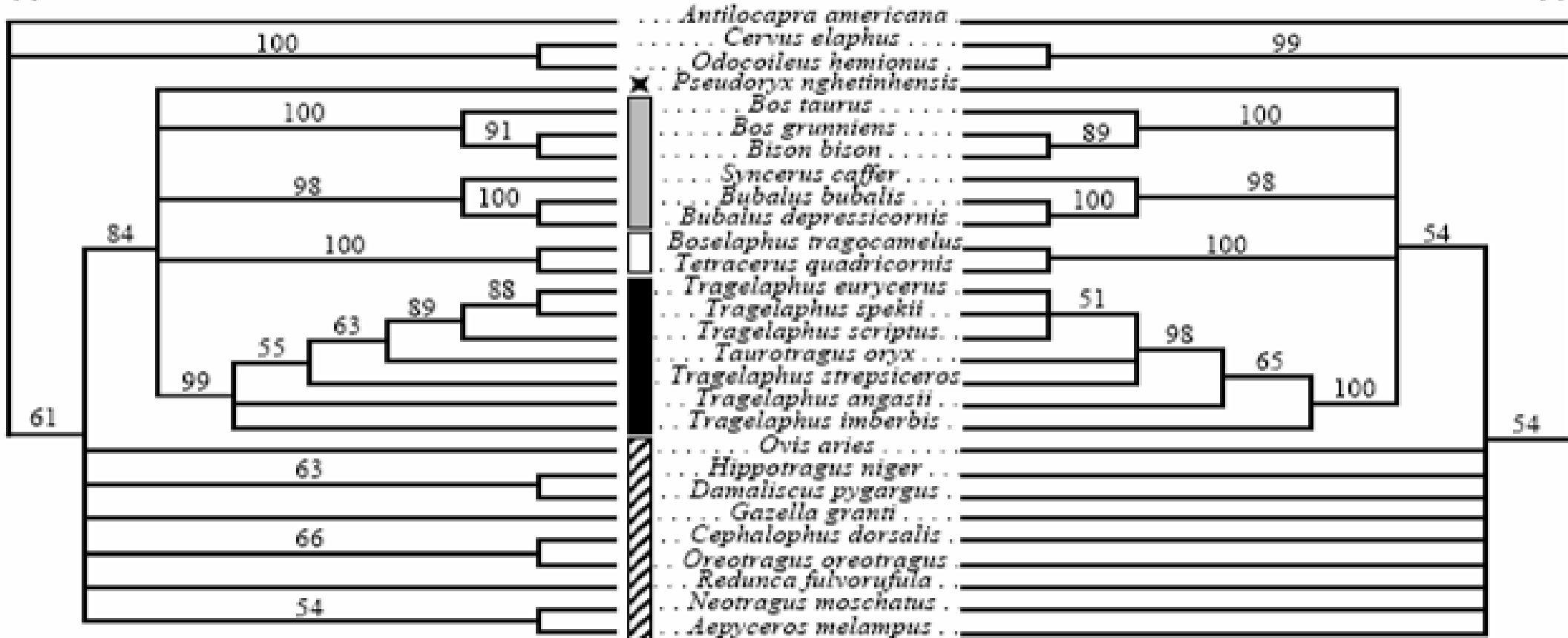
SAT I (blue)
 SAT III (red)
 SAT IV (green)



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

(a)

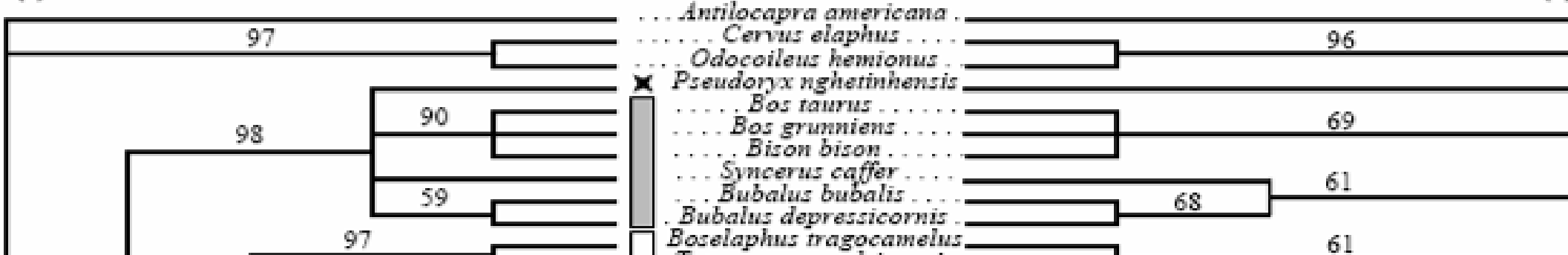
(b)

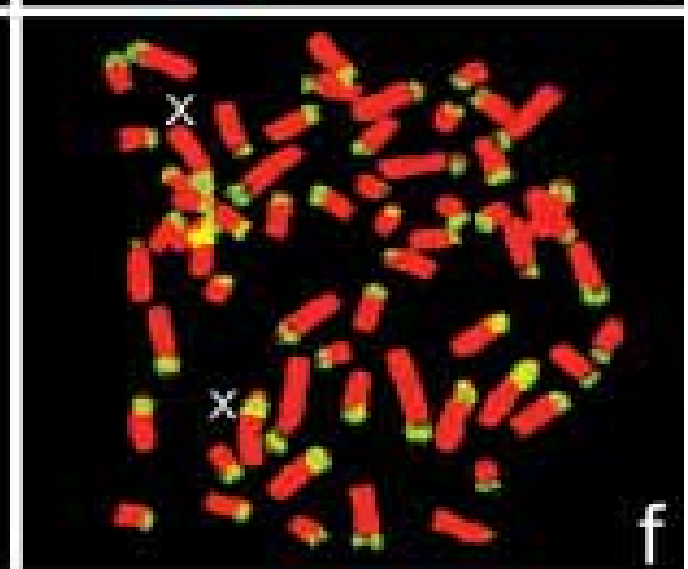
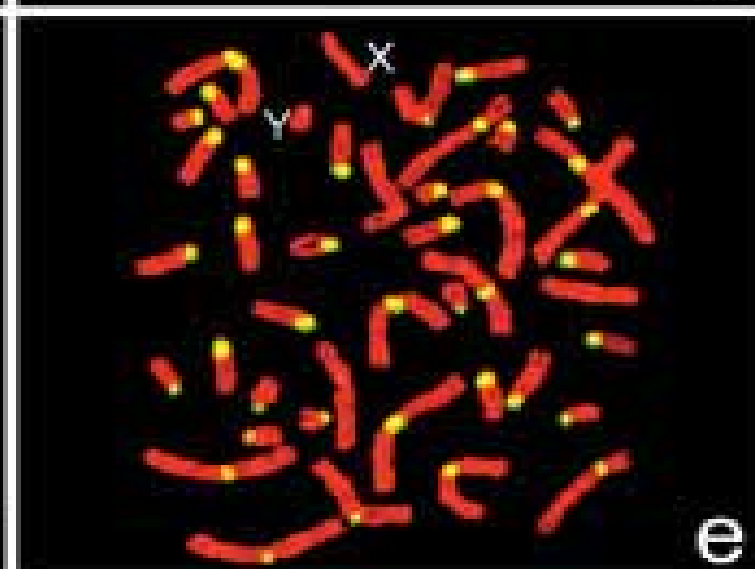
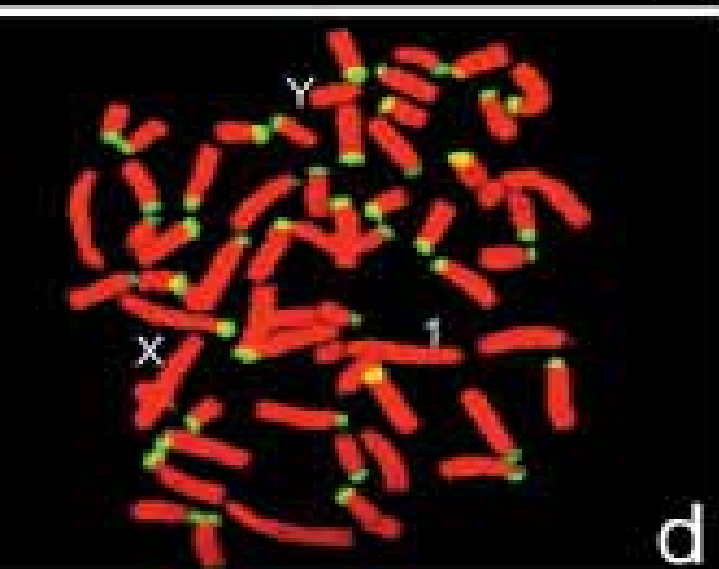
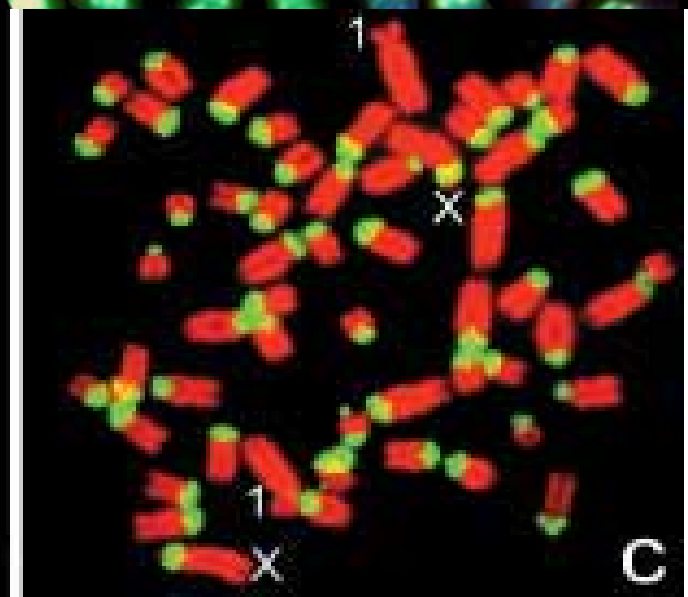
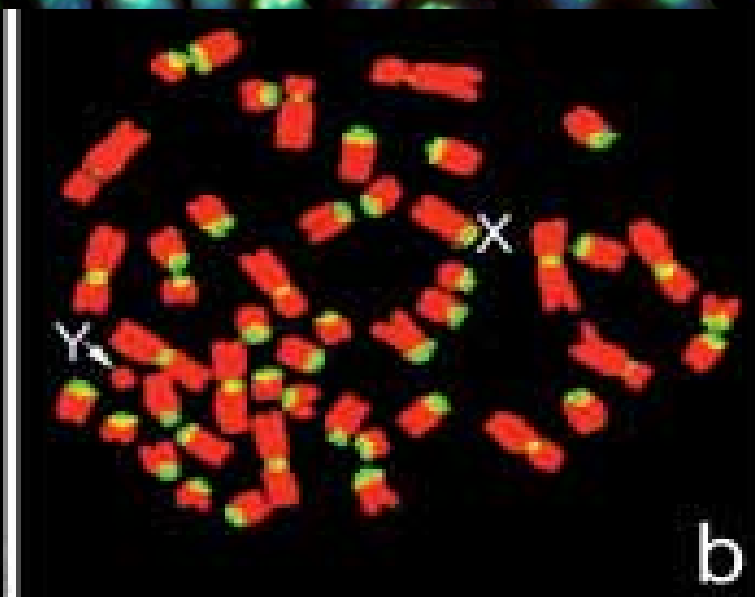
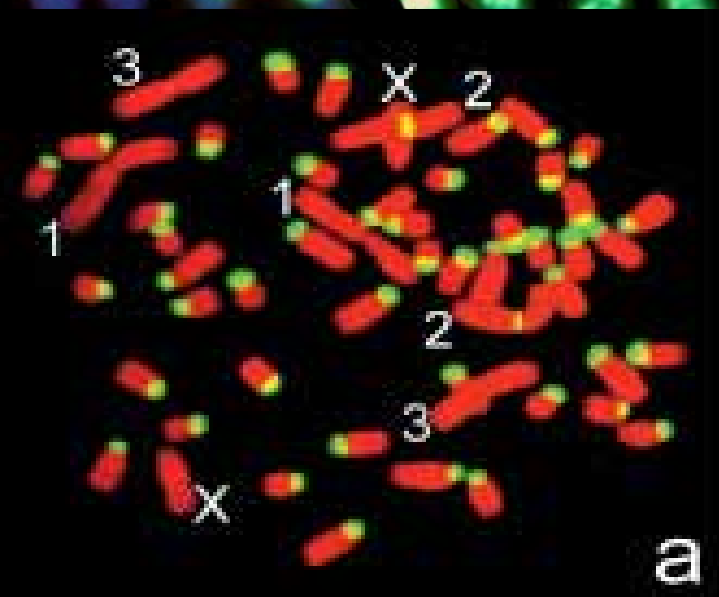


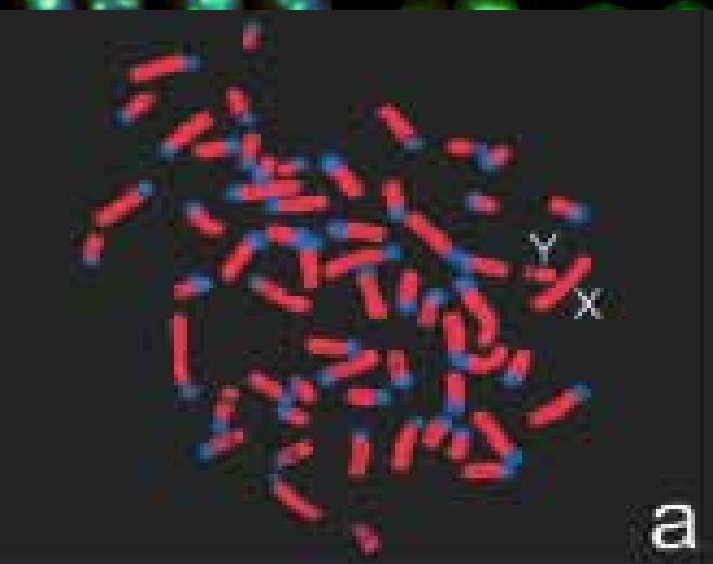
Hassanin & Douzery 1999 – Proc Roy Soc

(c)

(d)



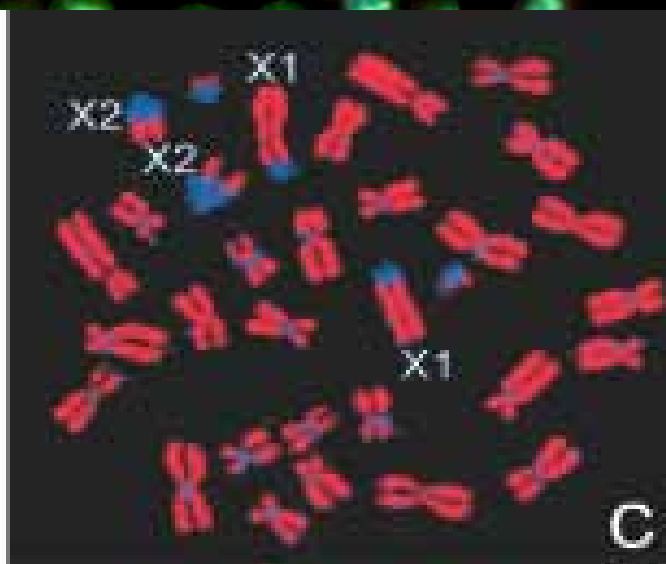




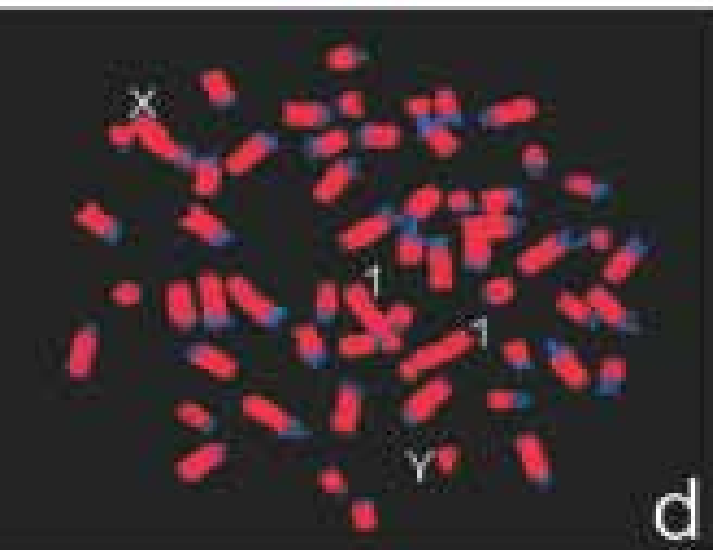
a



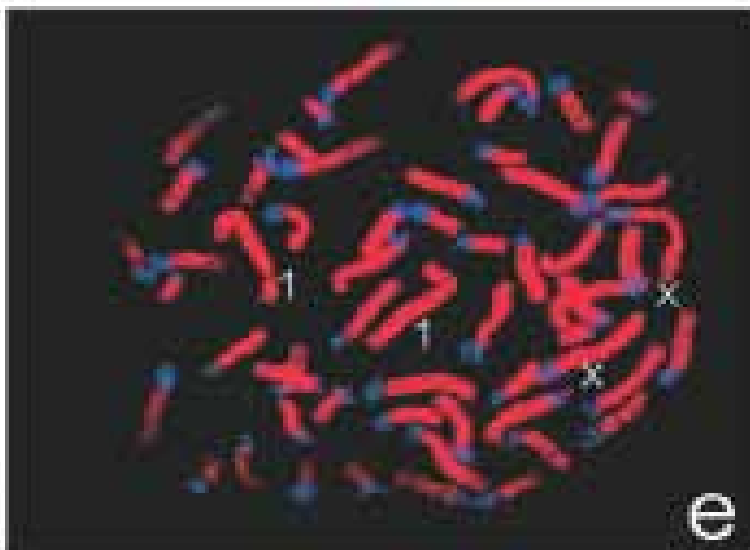
b



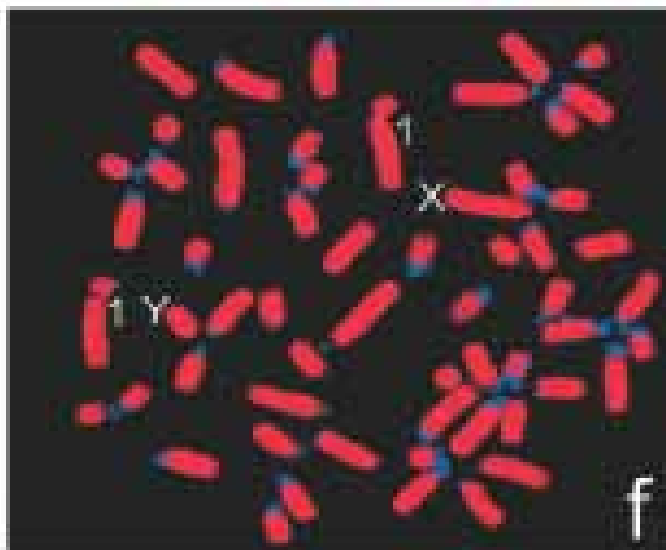
c



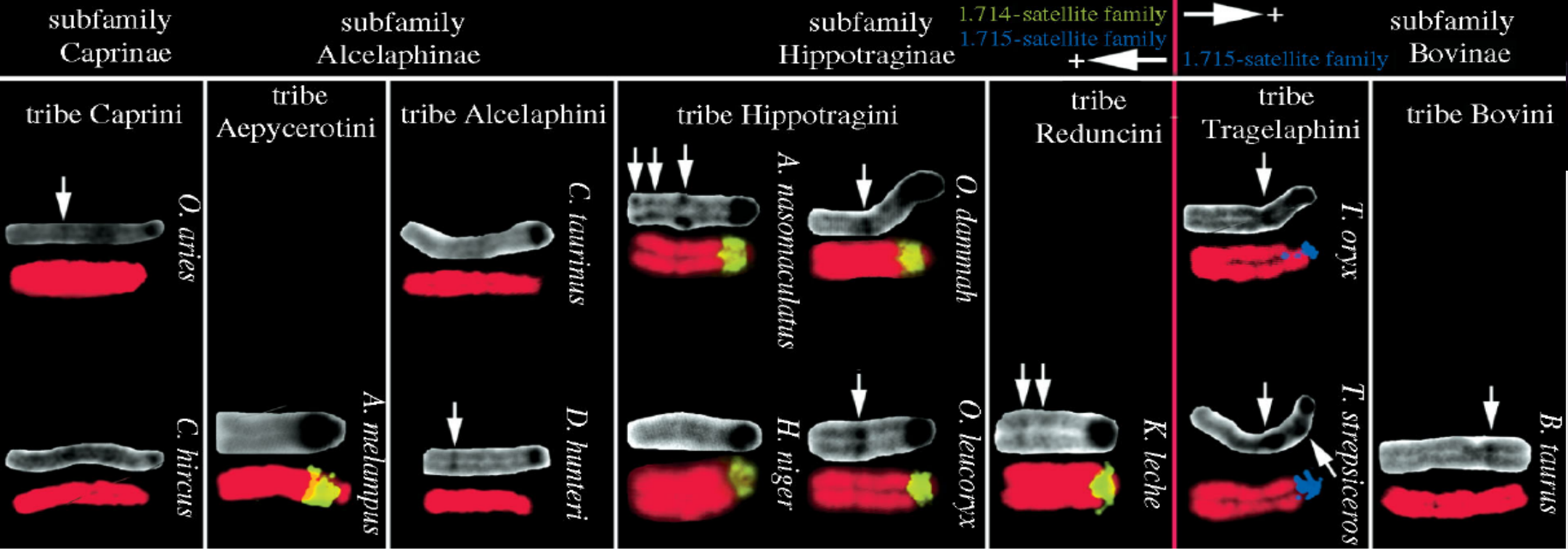
d



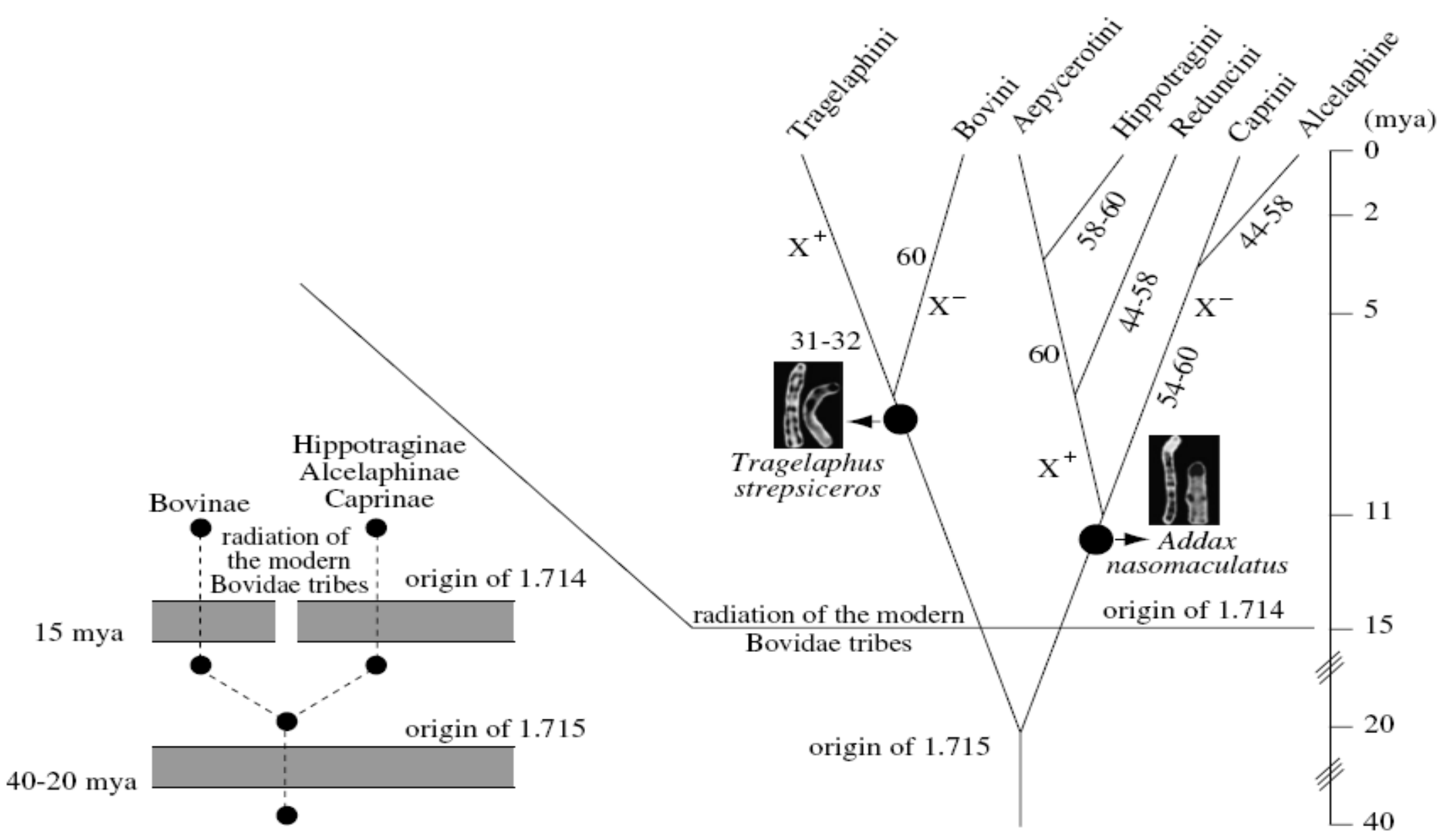
e



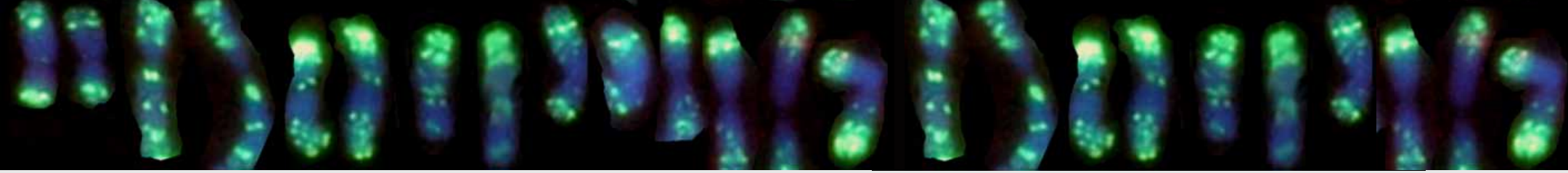
f



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

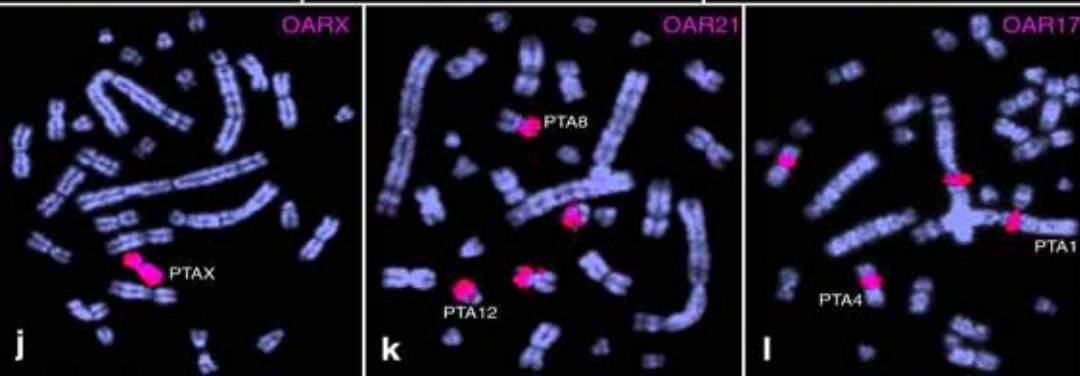
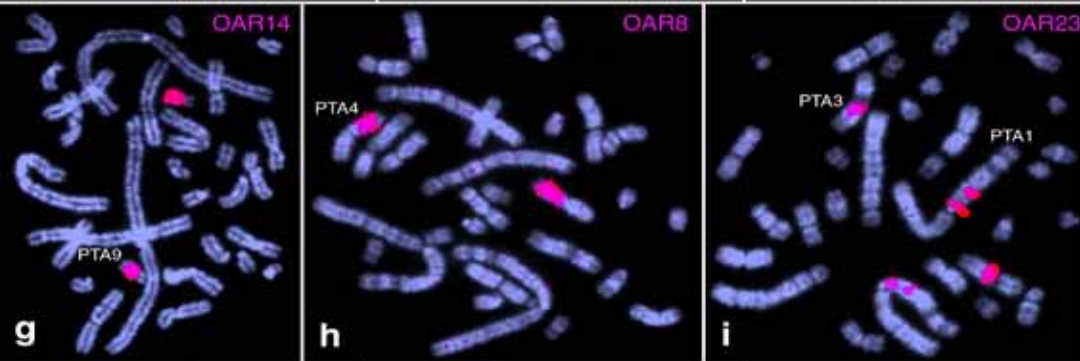
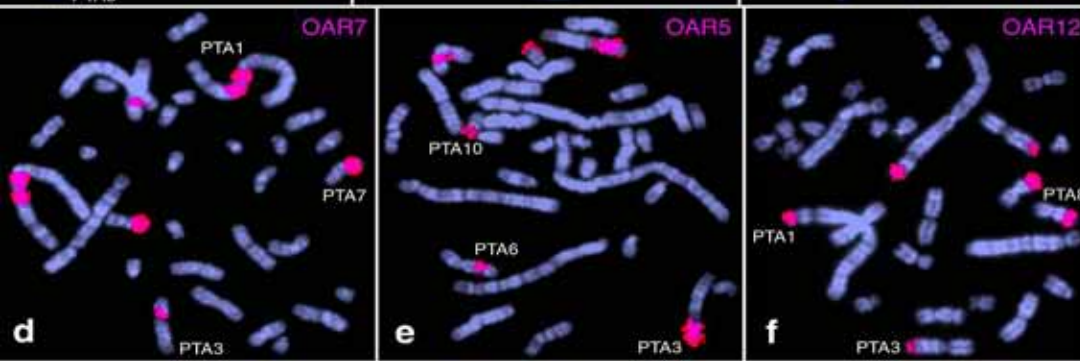
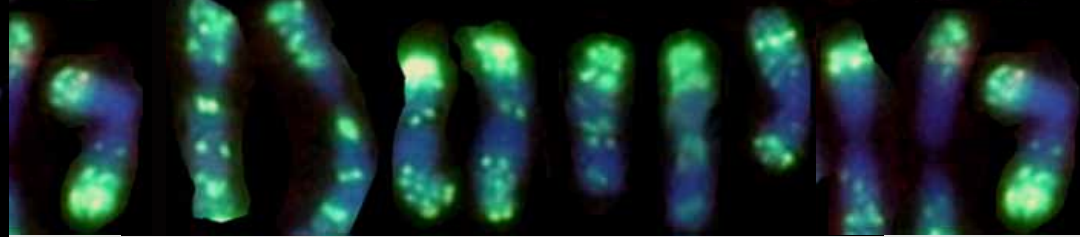
☞ Mammals

- 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

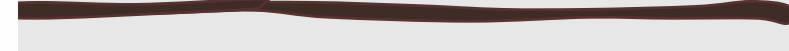
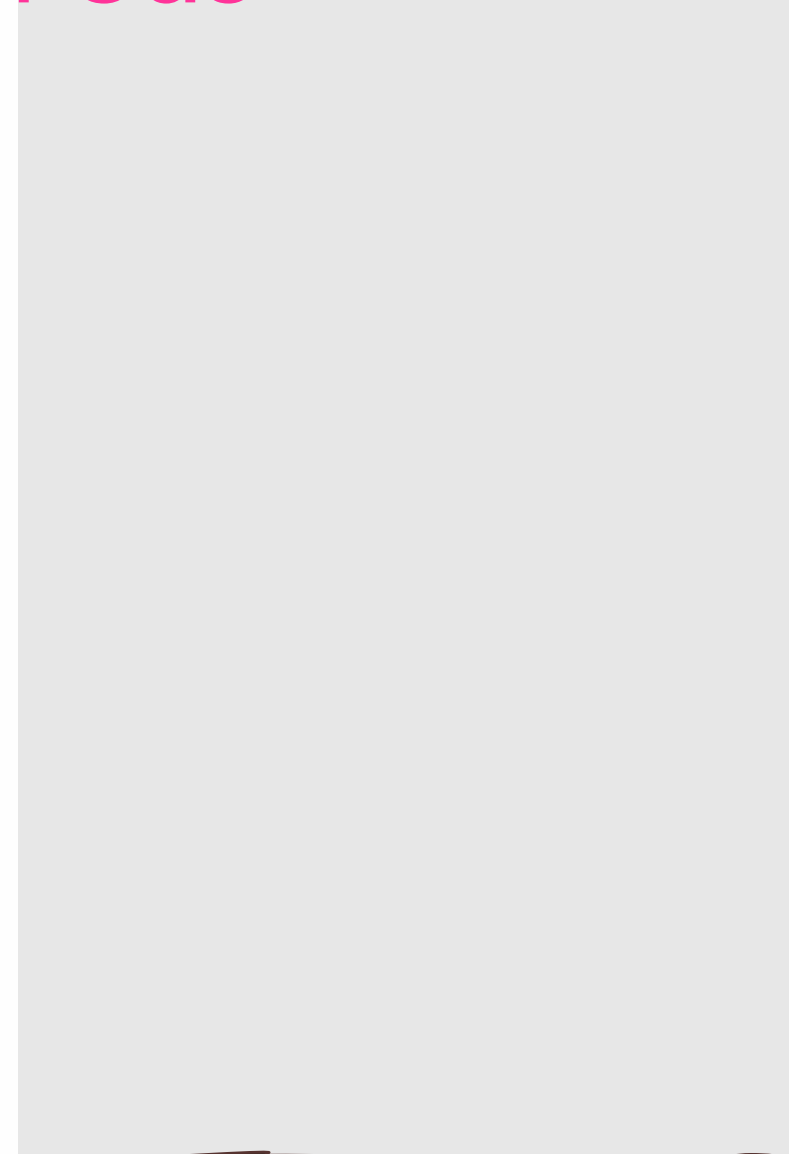
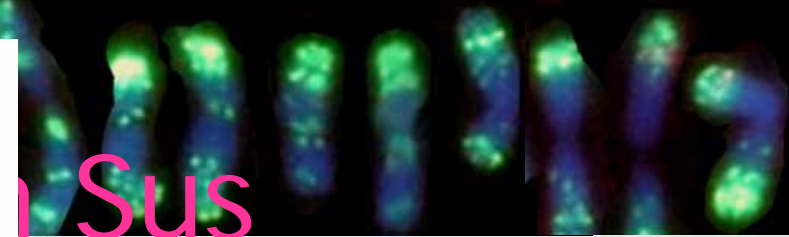
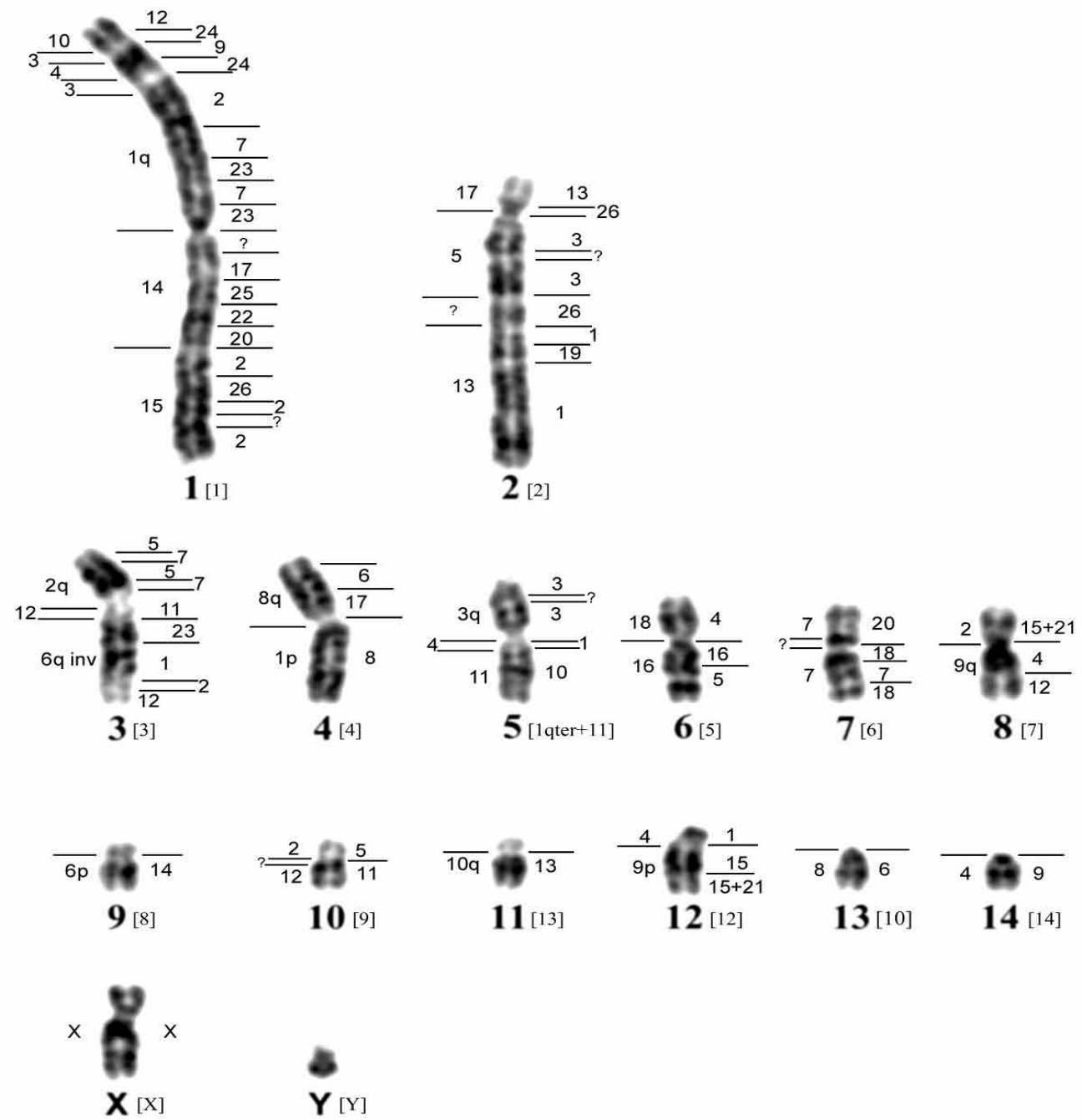


High-resolution comparative chromosome painting in the Arizona collared peccary (*Pecari tajacu*, *Tayassuidae*): a comparison with the karyotype of pig and sheep Filomena Adegá, Raquel Chaves, Andrea Kofler, Paul R. Krausman, Julio Masabanda, Johannes Wienberg & Henrique Guedes-Pinto. *Chromosome Research*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



Translocation in Sus

SSC OAR





What is a banana?

☛ Musa – Musaceae – Zingiberales

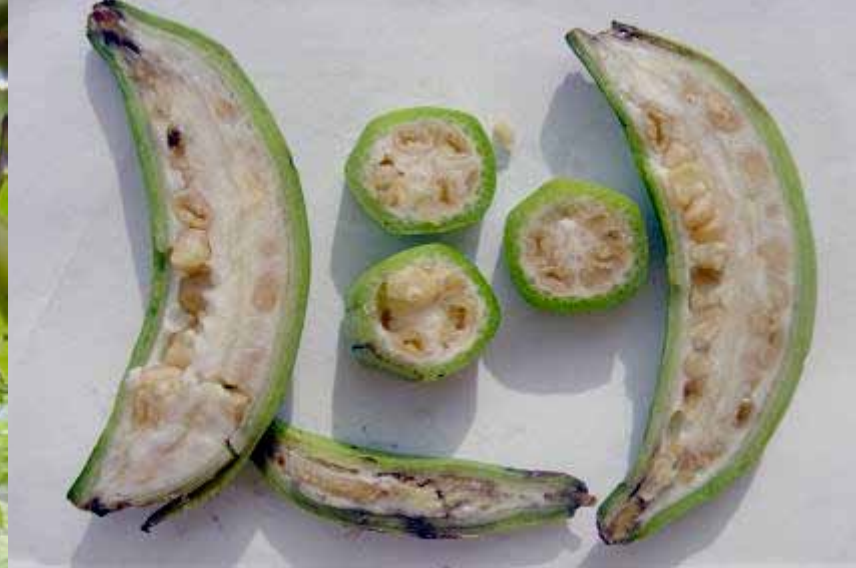
☛ Monocotyledon – Giant Herb (like grass not tree!)



Origins of the crop banana:

hybrids involving various diploids

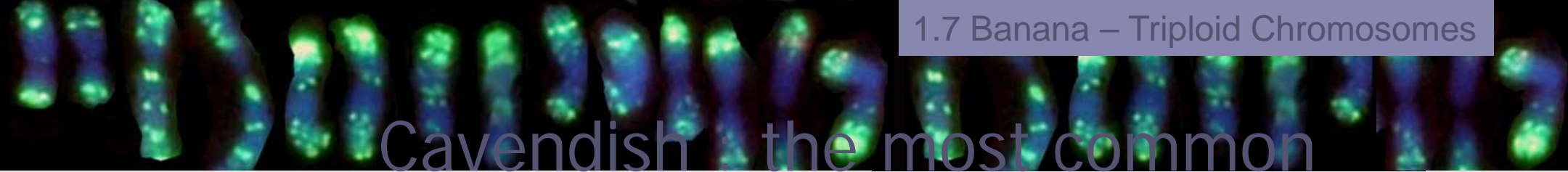
- ☛ The A genome from *Musa acuminata*
- ☛ The B genome from *Musa balbisiana*



Banana Plantains *Musa*

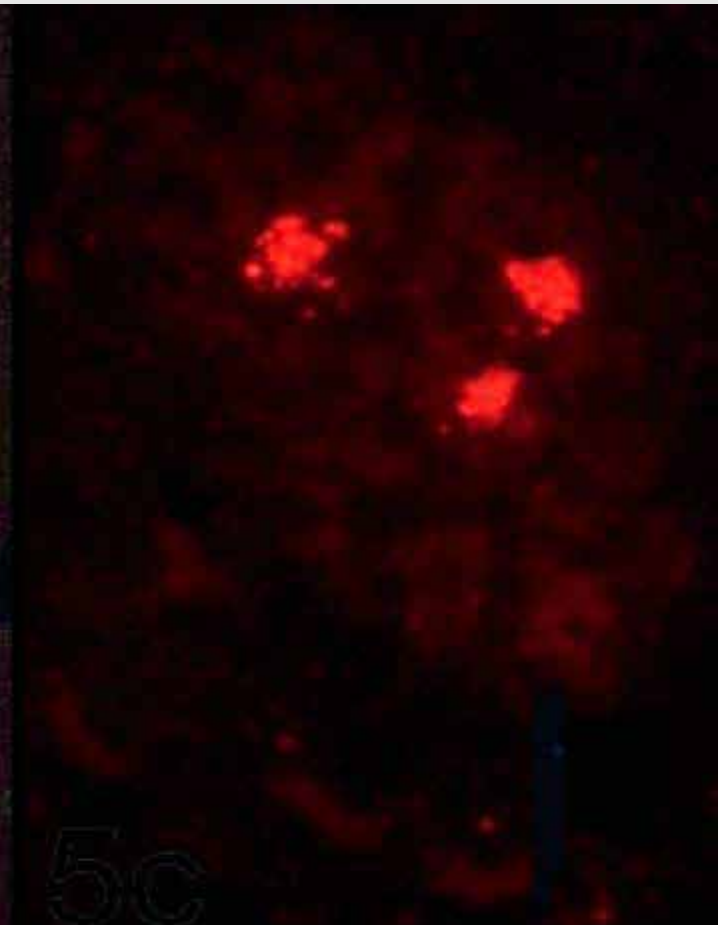
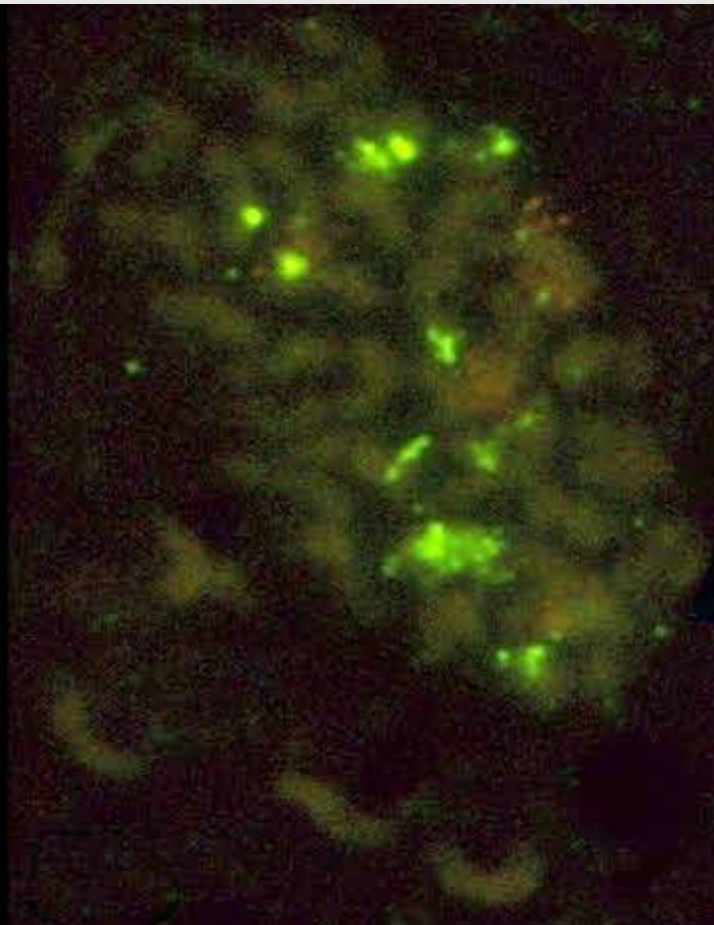
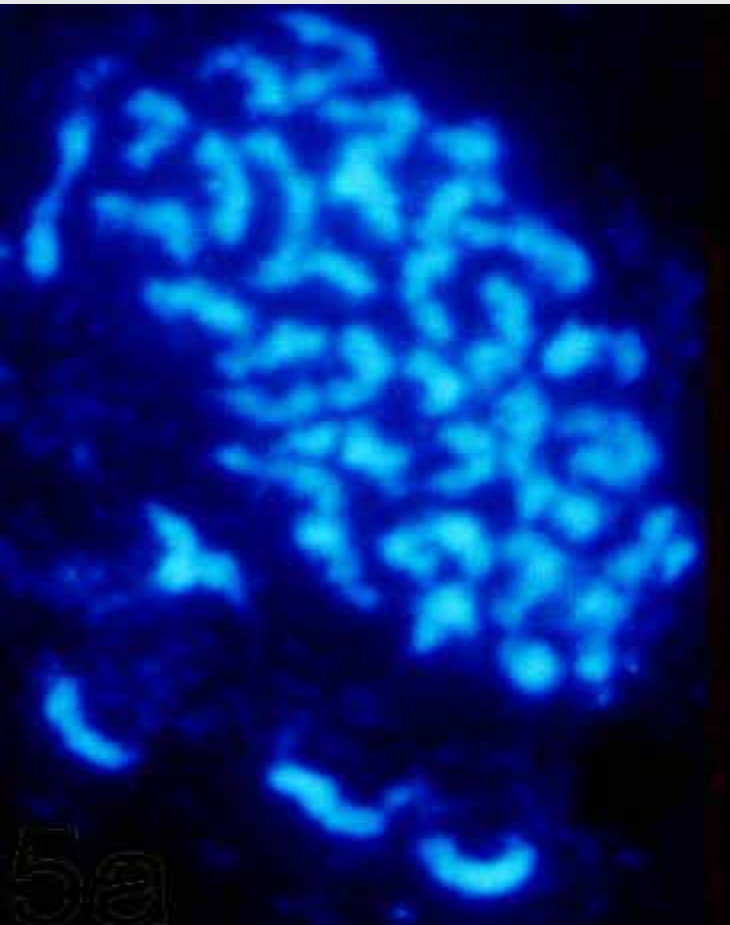






Cavendish : the most common
dessert banana cultivar

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



5a

5c



Rastali



Robusta



Palayamcodan



Red banana

Nendran



Monthan







Banana Genome Diversity

Cultivars are mostly parthenocarpic, sterile triploids, $2n=3x=33$

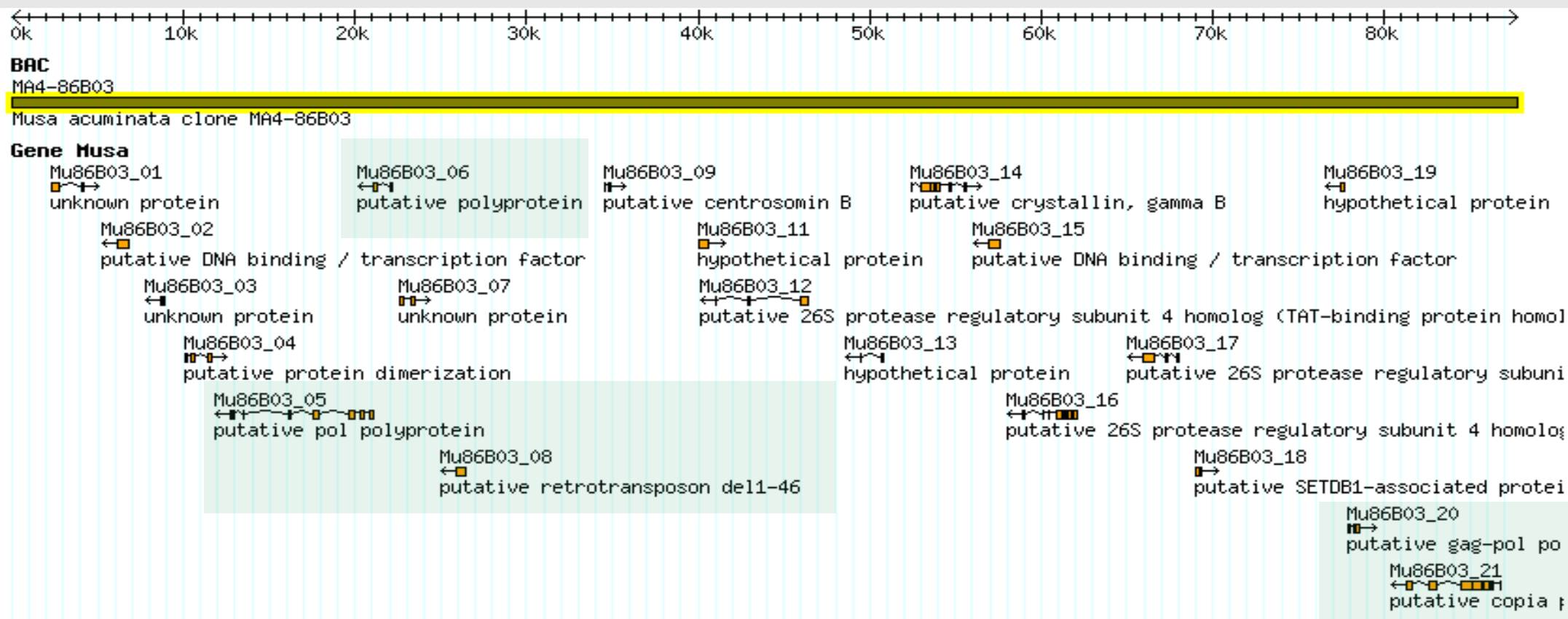
AAB and ABB plantains, cooking bananas



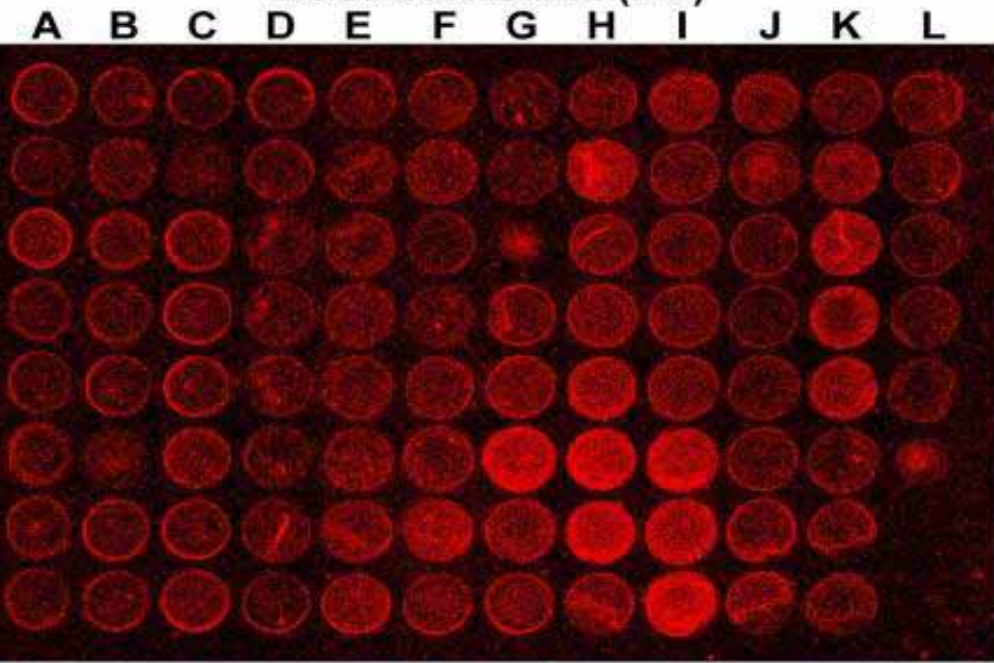
Retroelements

Sequences which amplify through an RNA intermediate

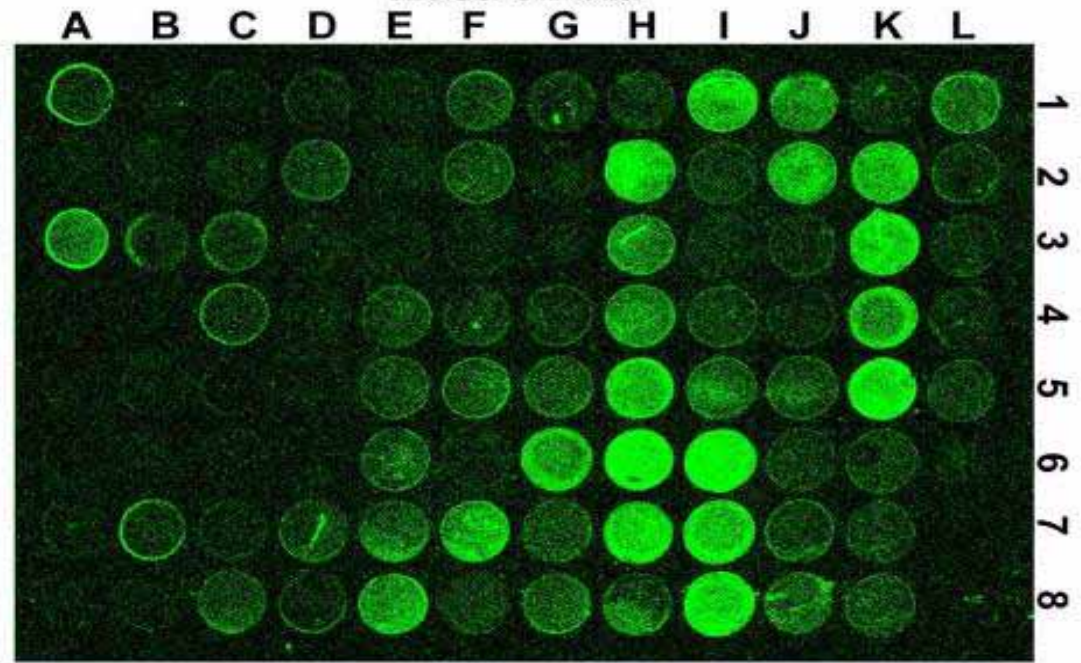
30% to 50% of all the DNA!



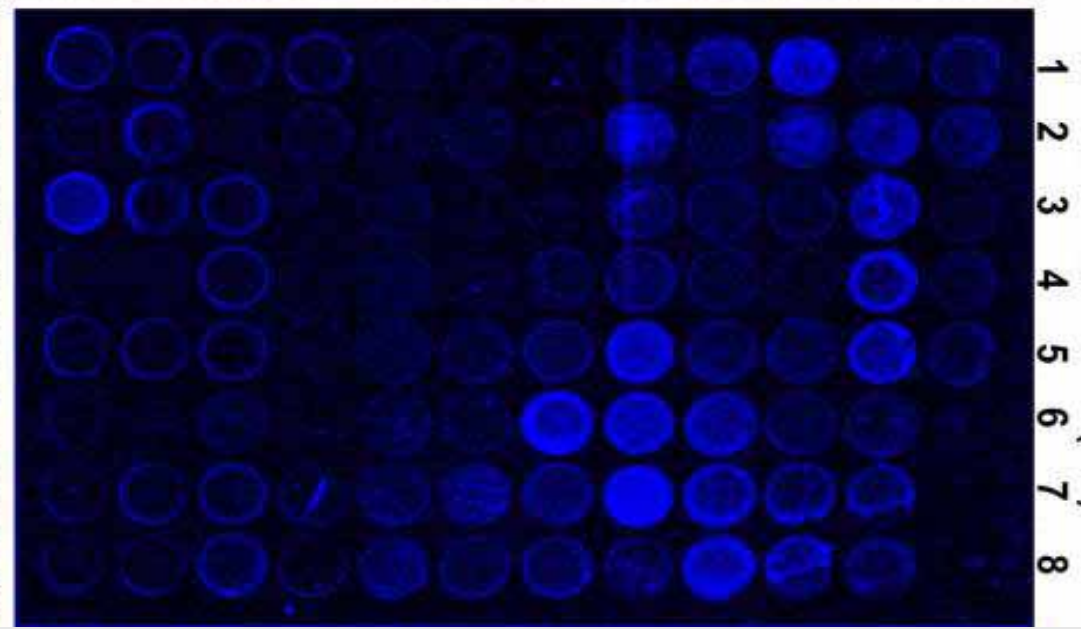
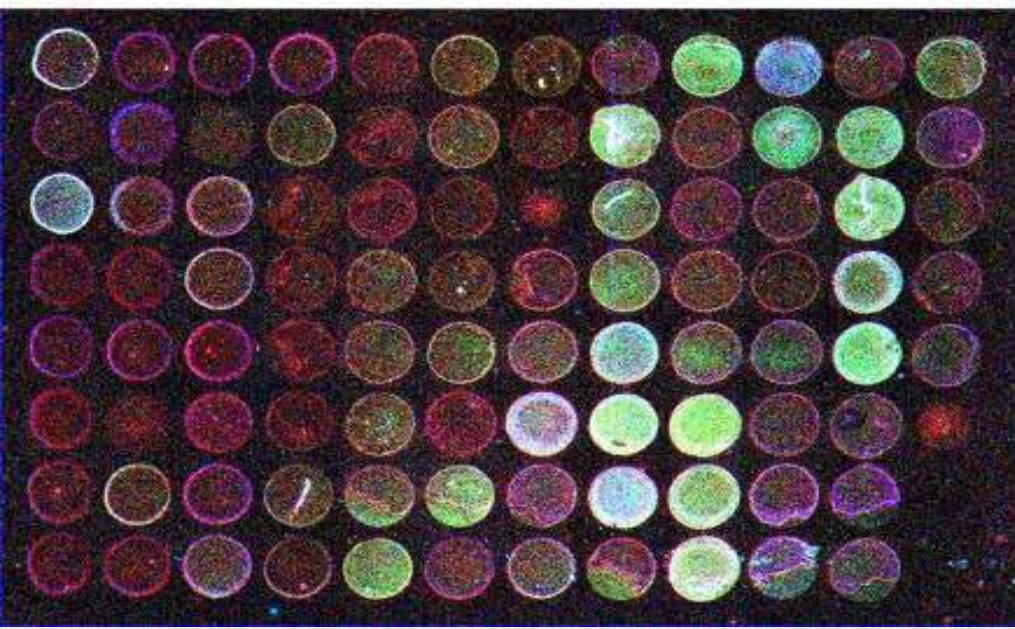
Musa balbisiana (BB)



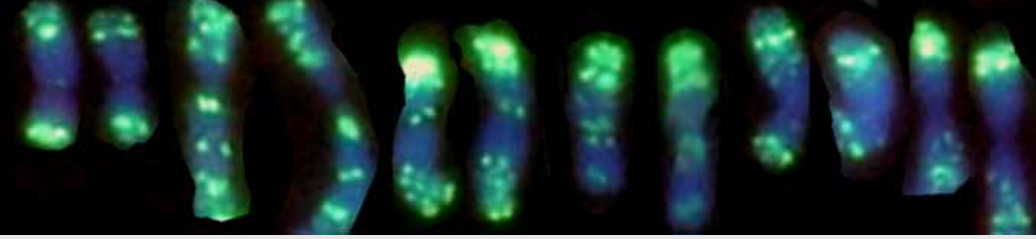
Musa ornata



1
2
3
4
5
6
7
8



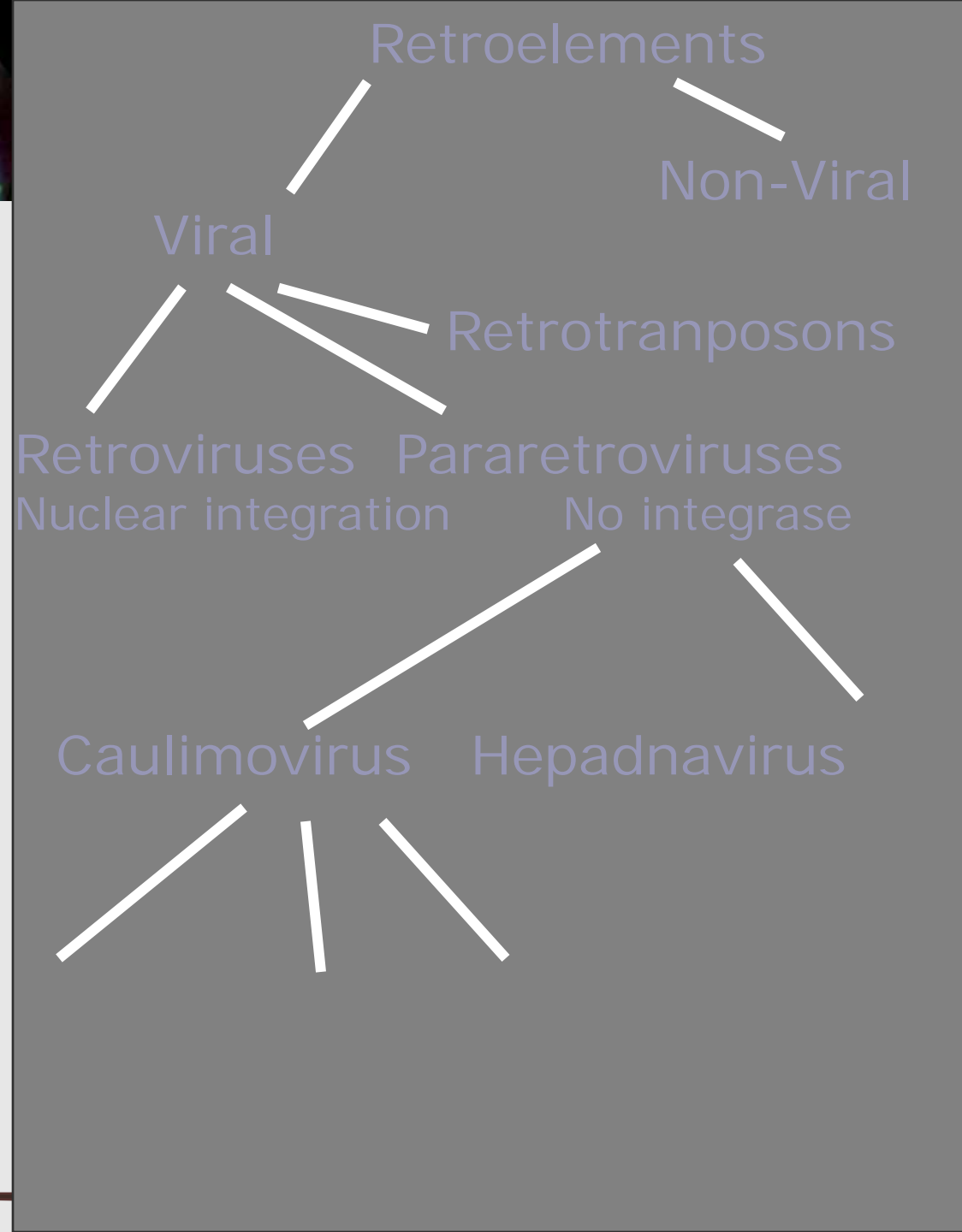
Musa acuminata (AA)
1
2
3
4
5
6
7
8



Major Repetitive Sequences

- ☛ Tandem Repeats
- ☛ Simple Sequence Repeats
- ☛ Dispersed Repeats
- ☛ Functional Repeats
- ☛ Retroelements

See Hansen & HH 2004





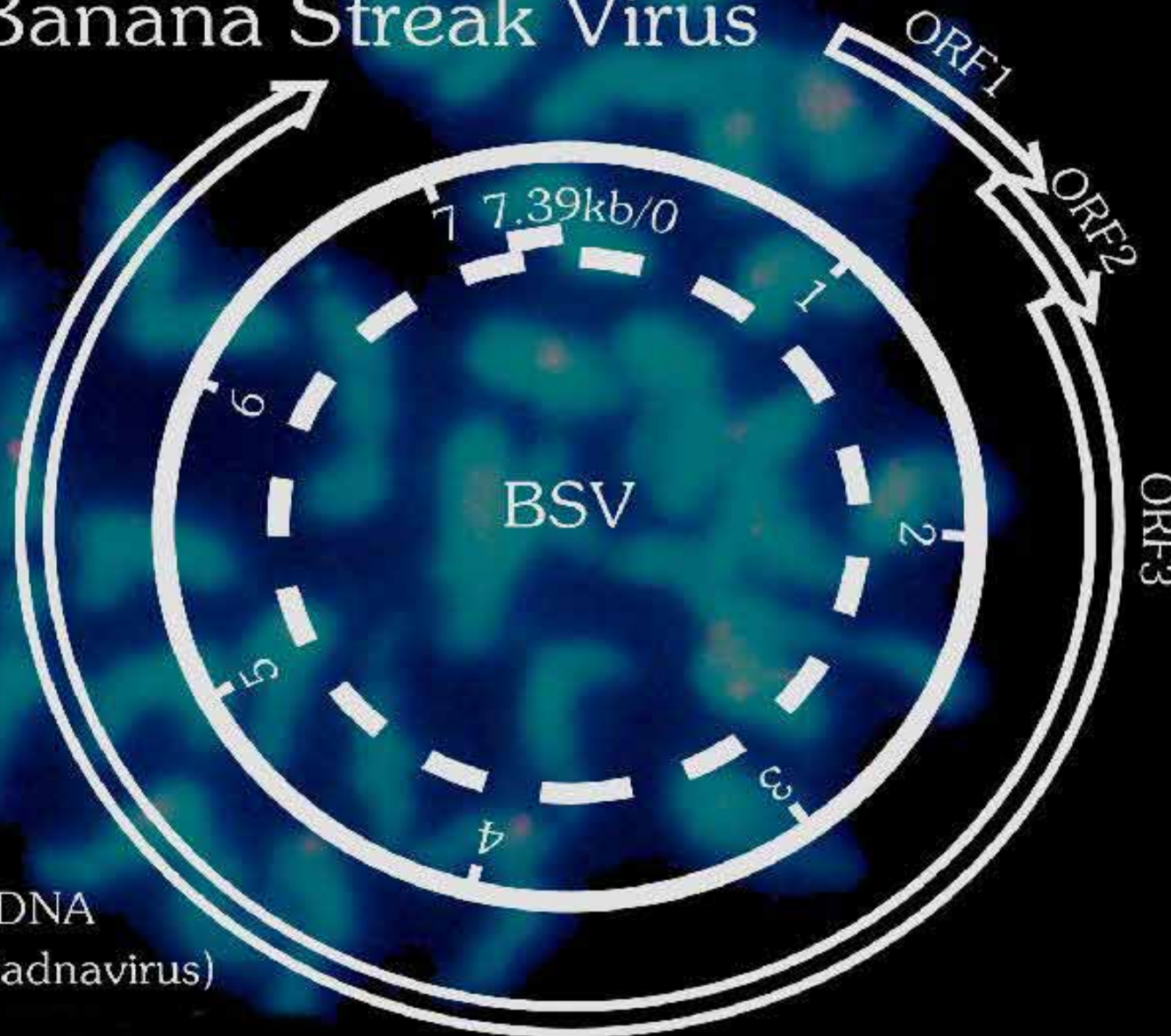
Banana Streak ParaRetrovirus (BSV)

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

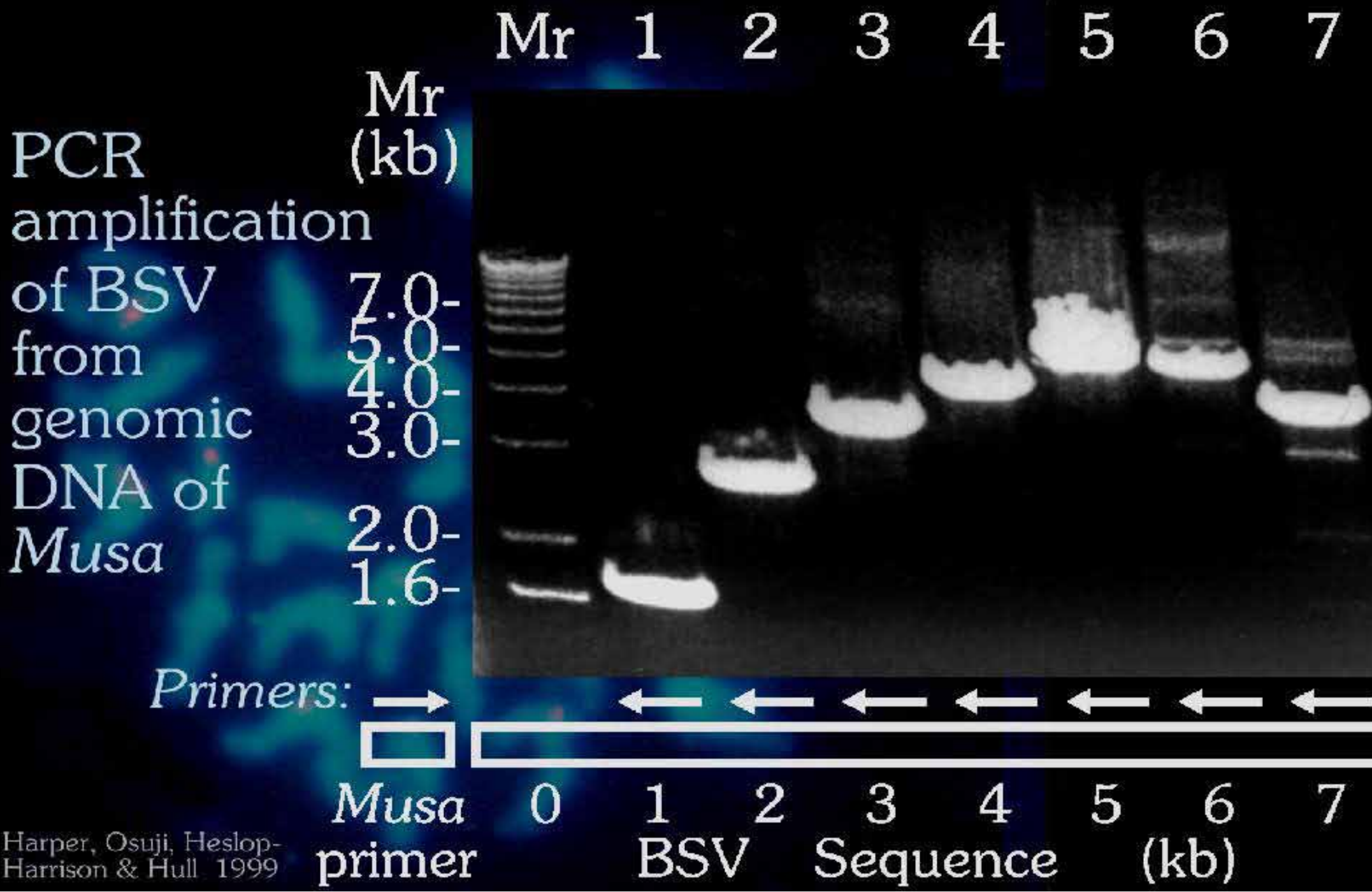
Glyn Harper & Roger Hull



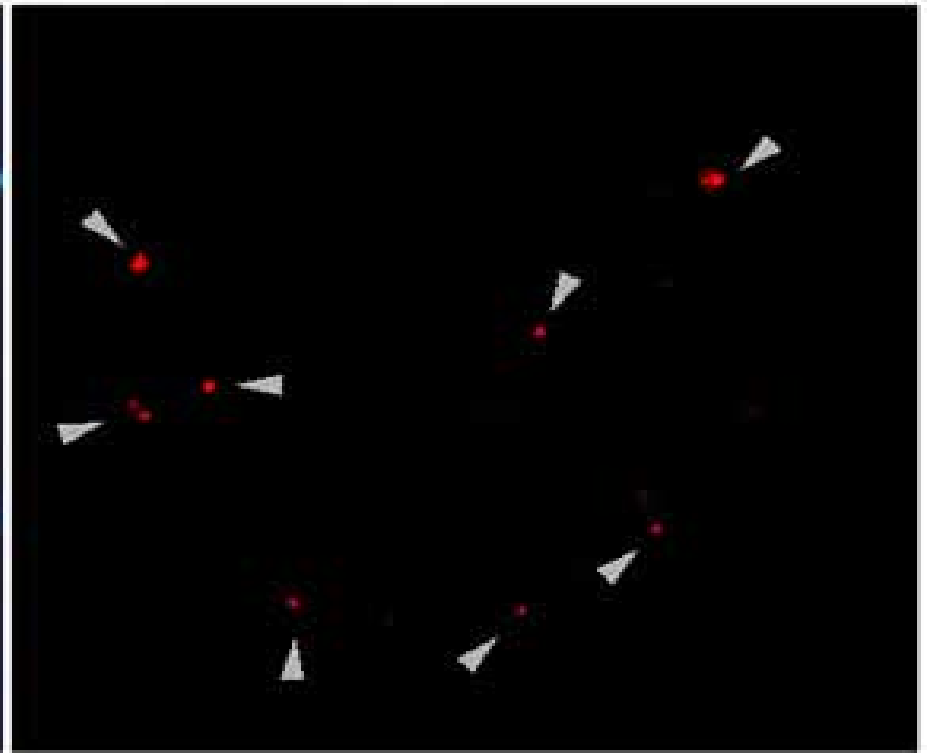
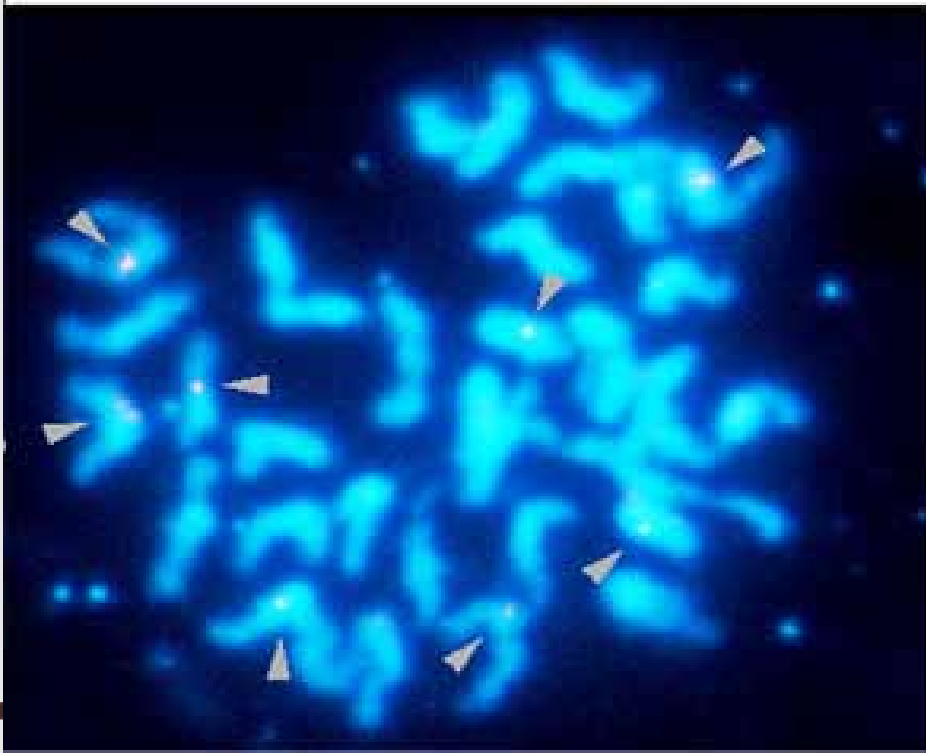
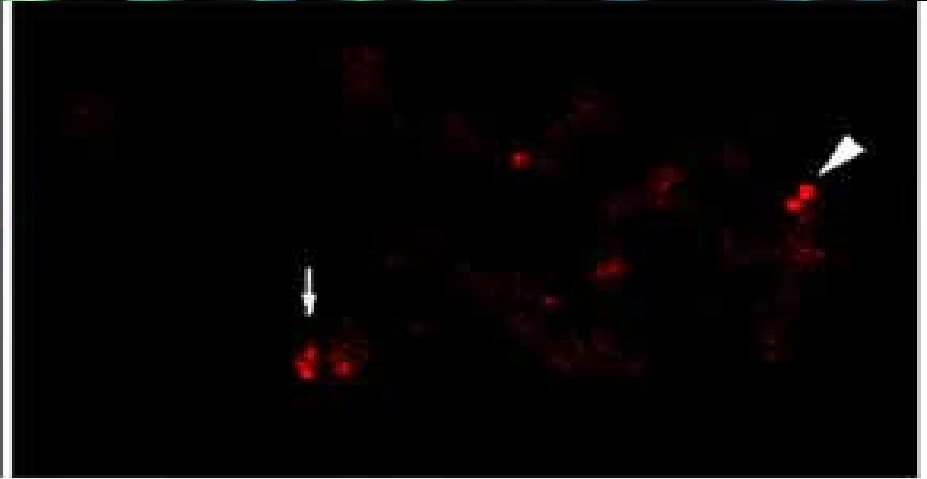
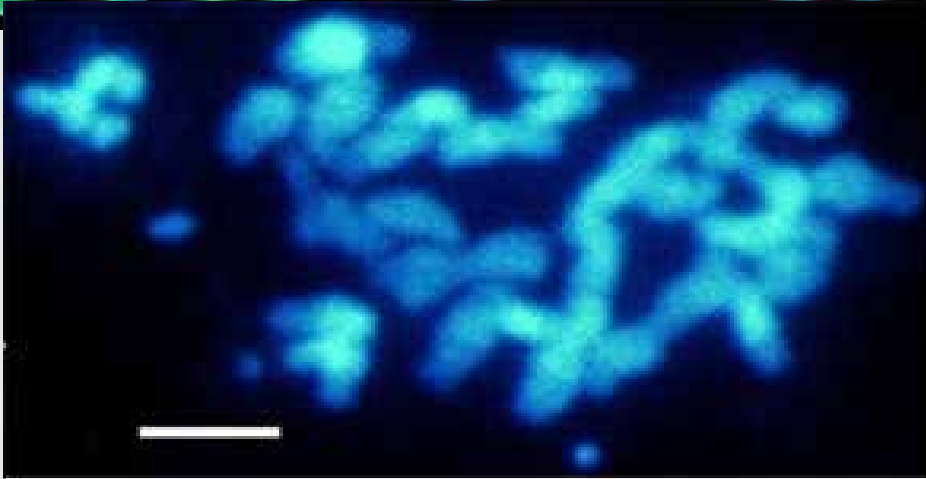
Banana Streak Virus



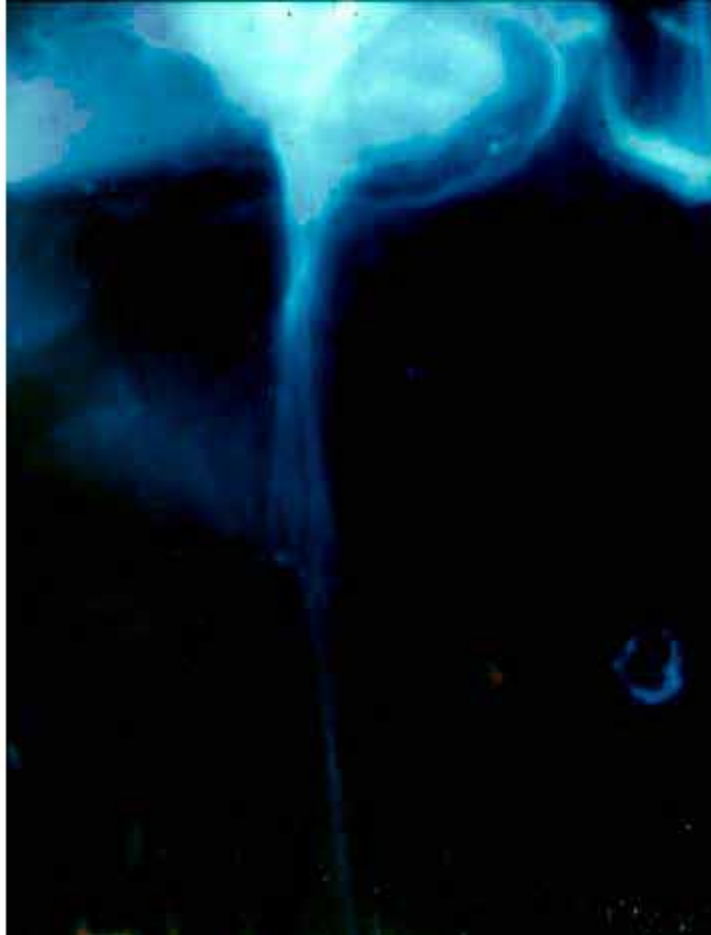
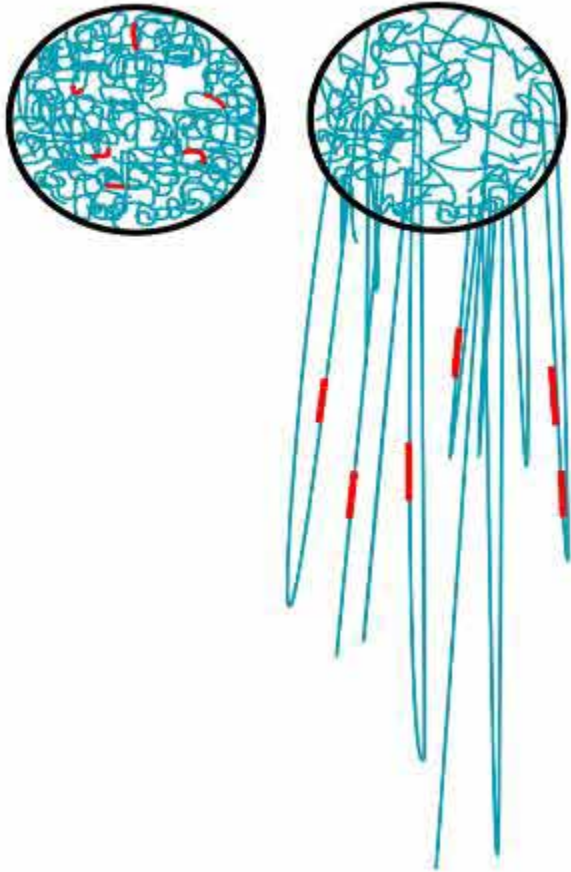
Double-stranded DNA
ParaRetrovirus (Badnavirus)



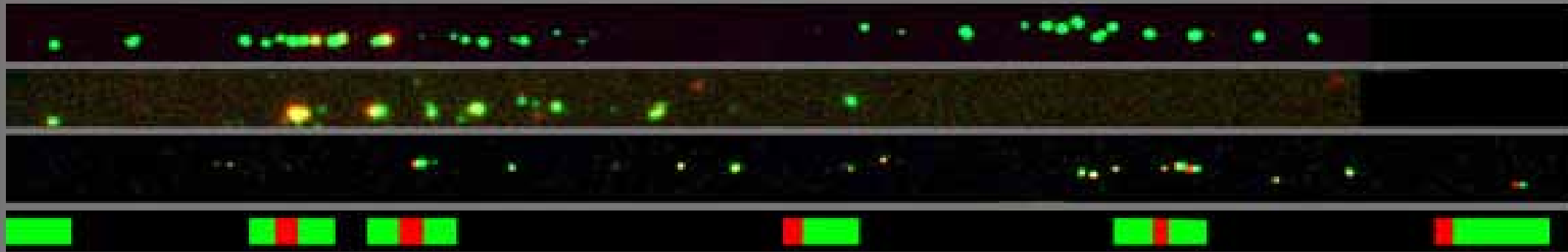
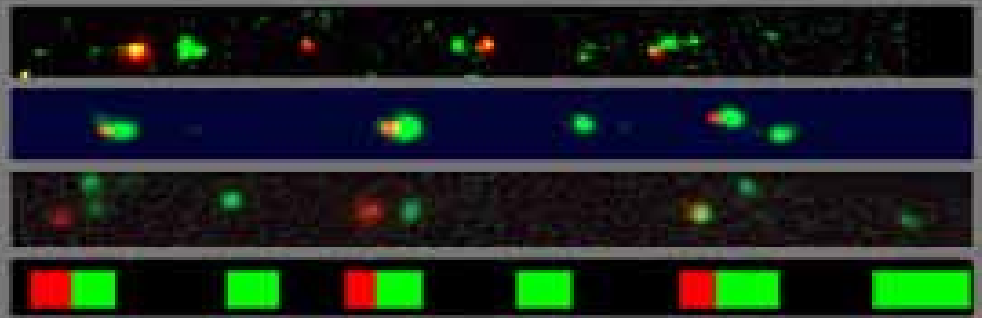
Nuclear Copies of BSV in Banana



DNA Fibre Hybridization



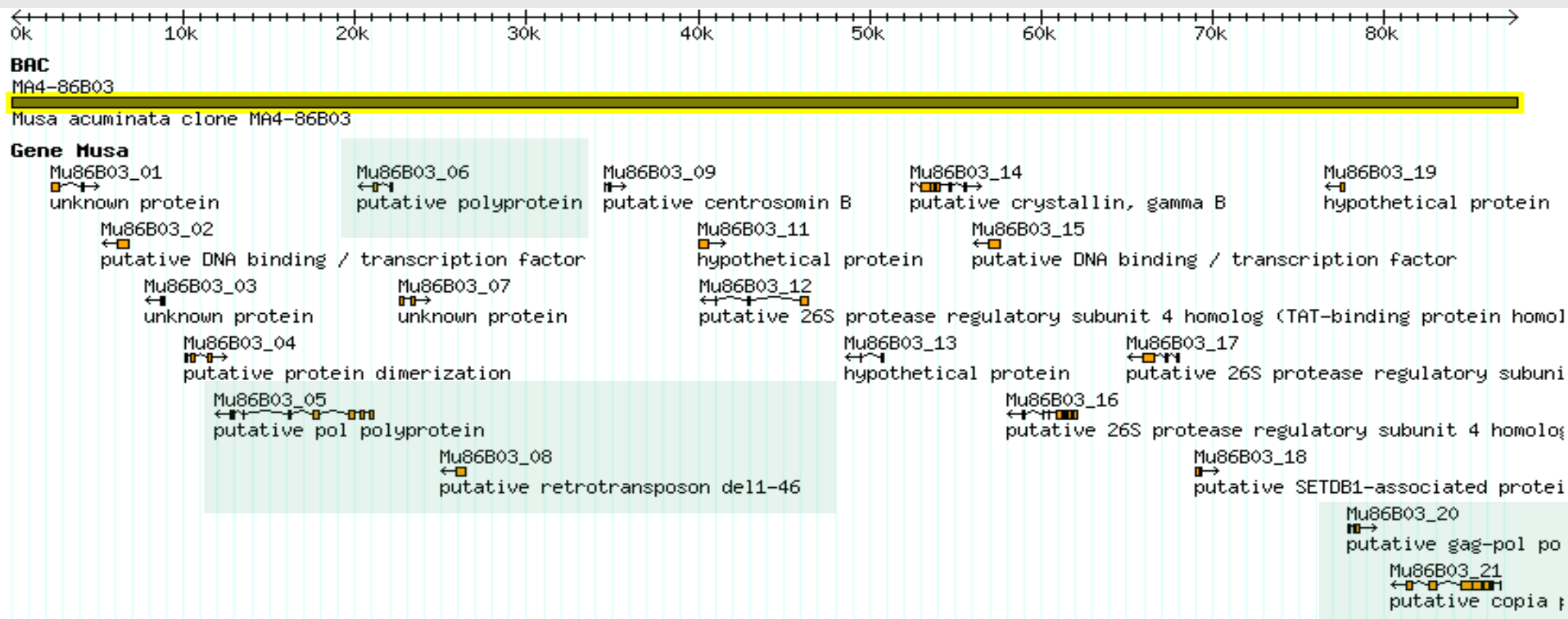
Nuclear Copies of BSV in Banana



Retroelements

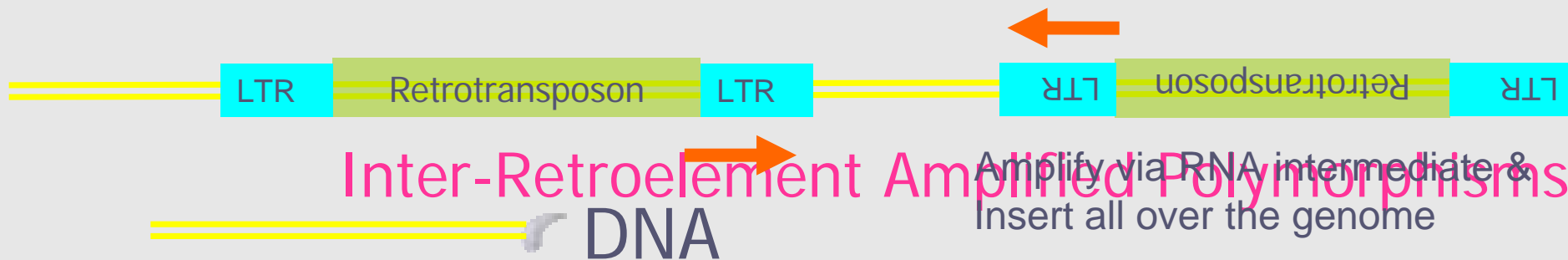
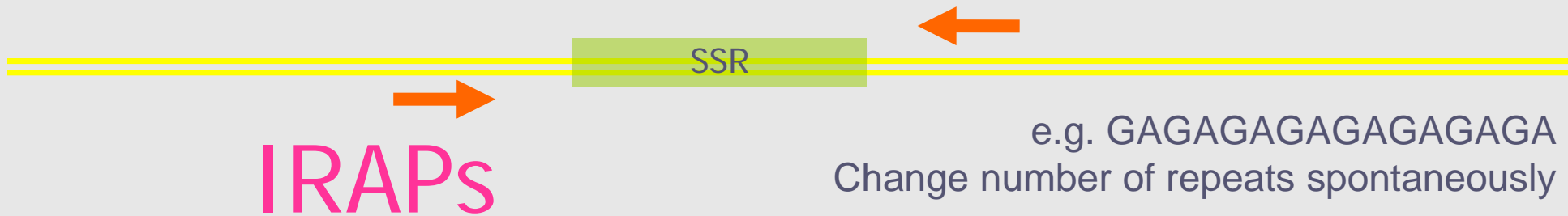
Sequences which amplify through an RNA intermediate

30% to 50% of all the DNA!

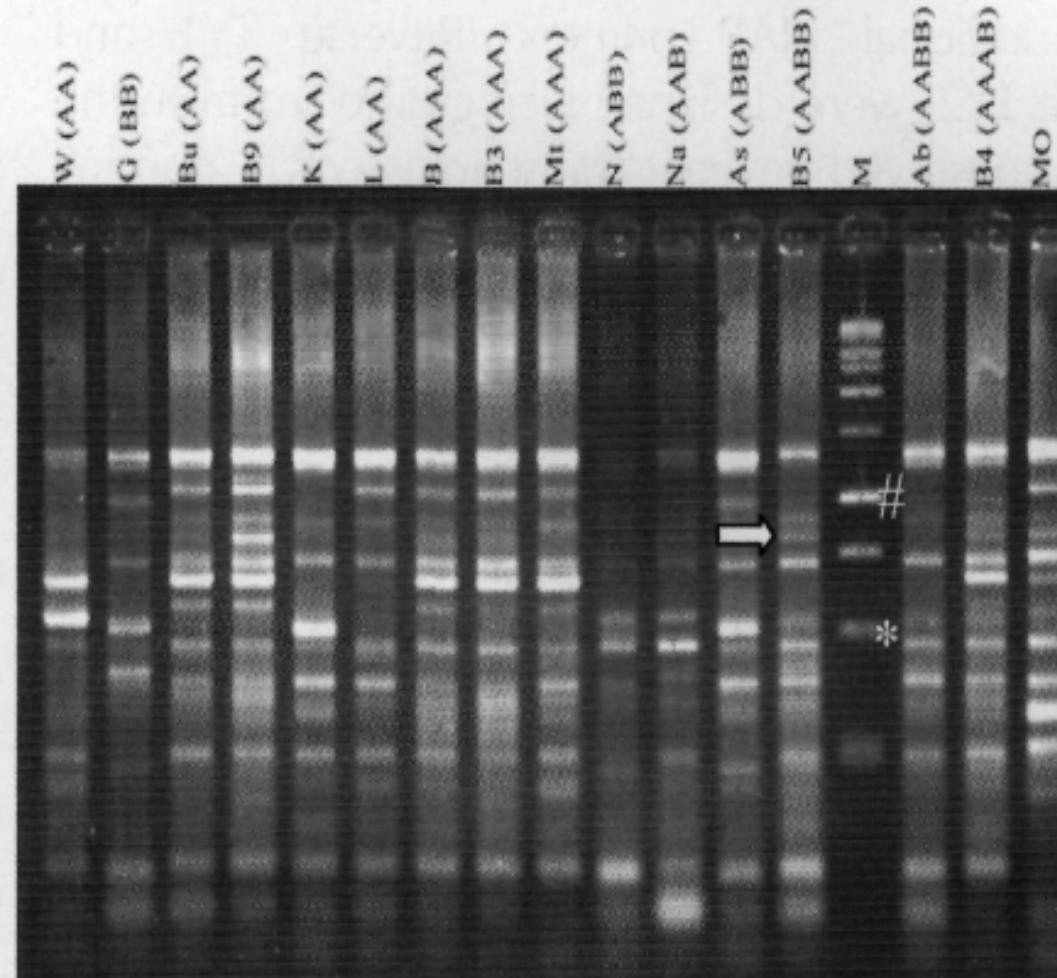
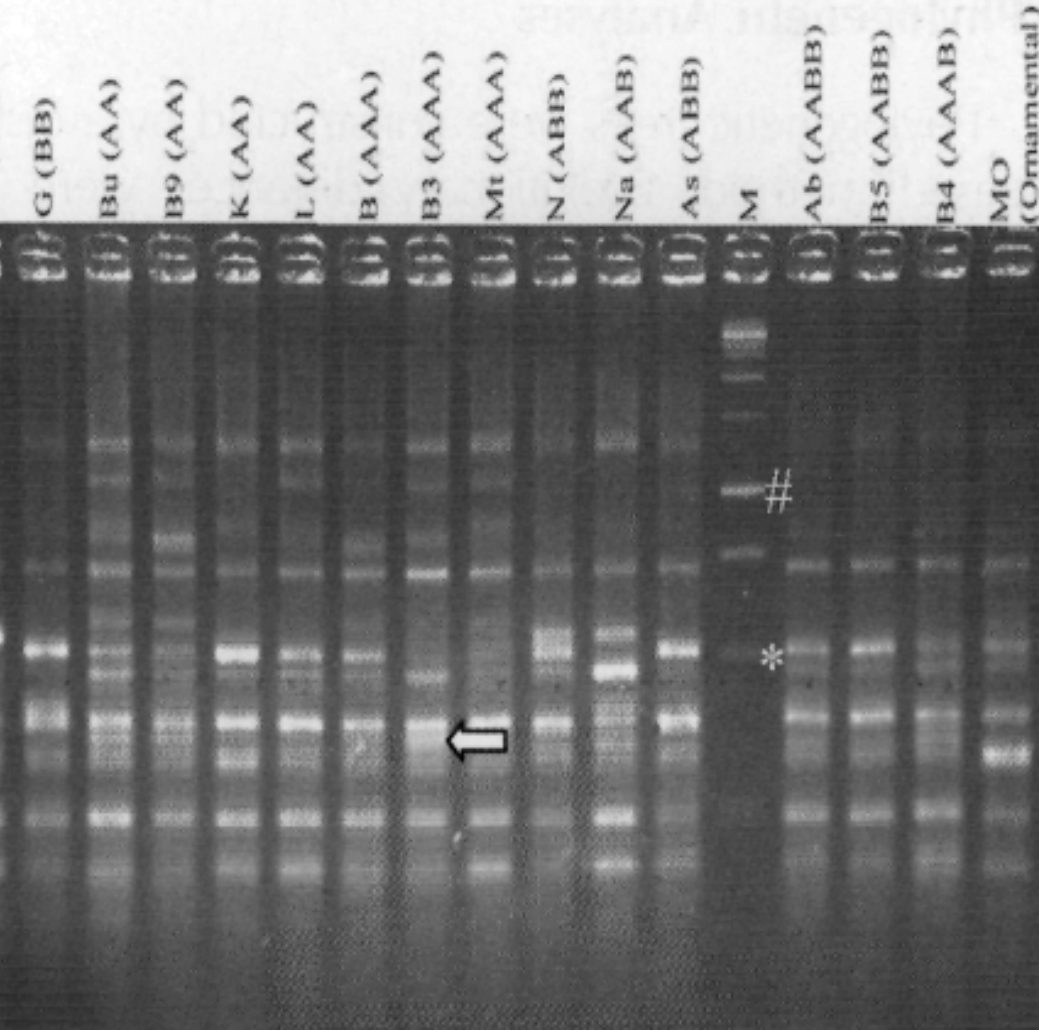


diversity and relationships

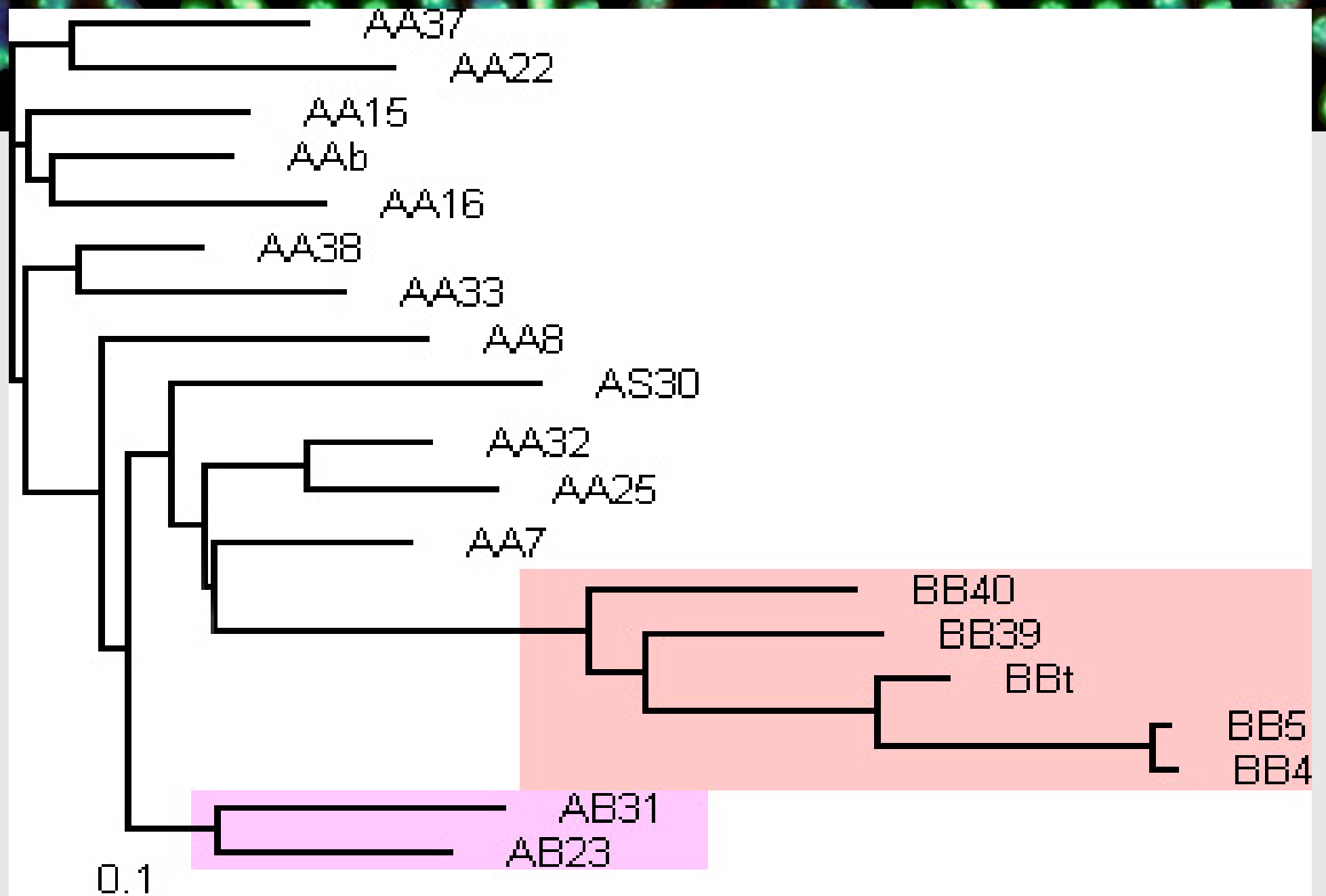
Microsatellites or simple sequence repeats



IRAP diversity in Musa



Teo, Tan, Ho, Faridah, Othman, HH, Kalendar, Schulman 2005 *J Plant Biol*
Nair, Teo, Schwarzacher, HH 2005 *Euphytica*
Desai, Maha..., HH et al. in prep.

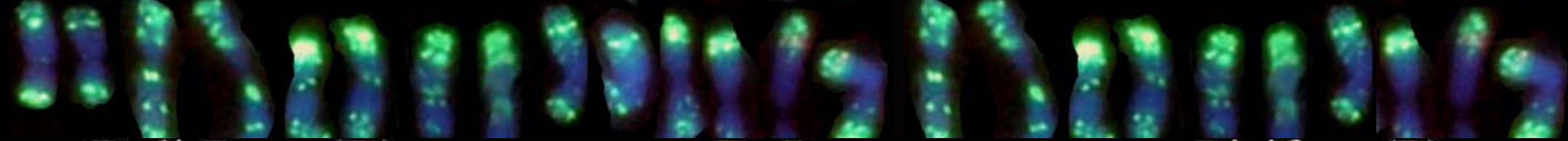


Oil palm: *Elaeis guineensis*



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

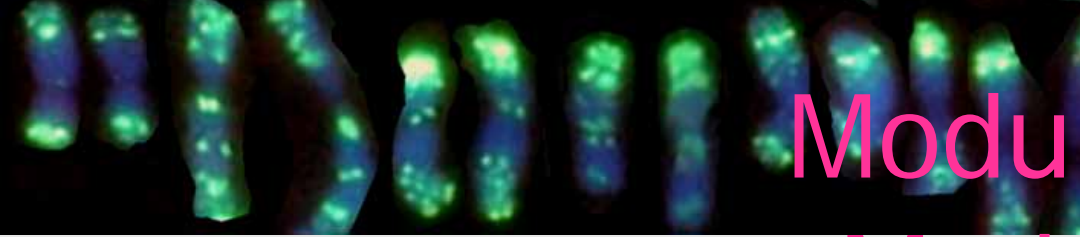
McrBC

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

Cuts ^mC NNNNNNNNNNNN ^mC

Leaves C NNNNNNNNNNNN C

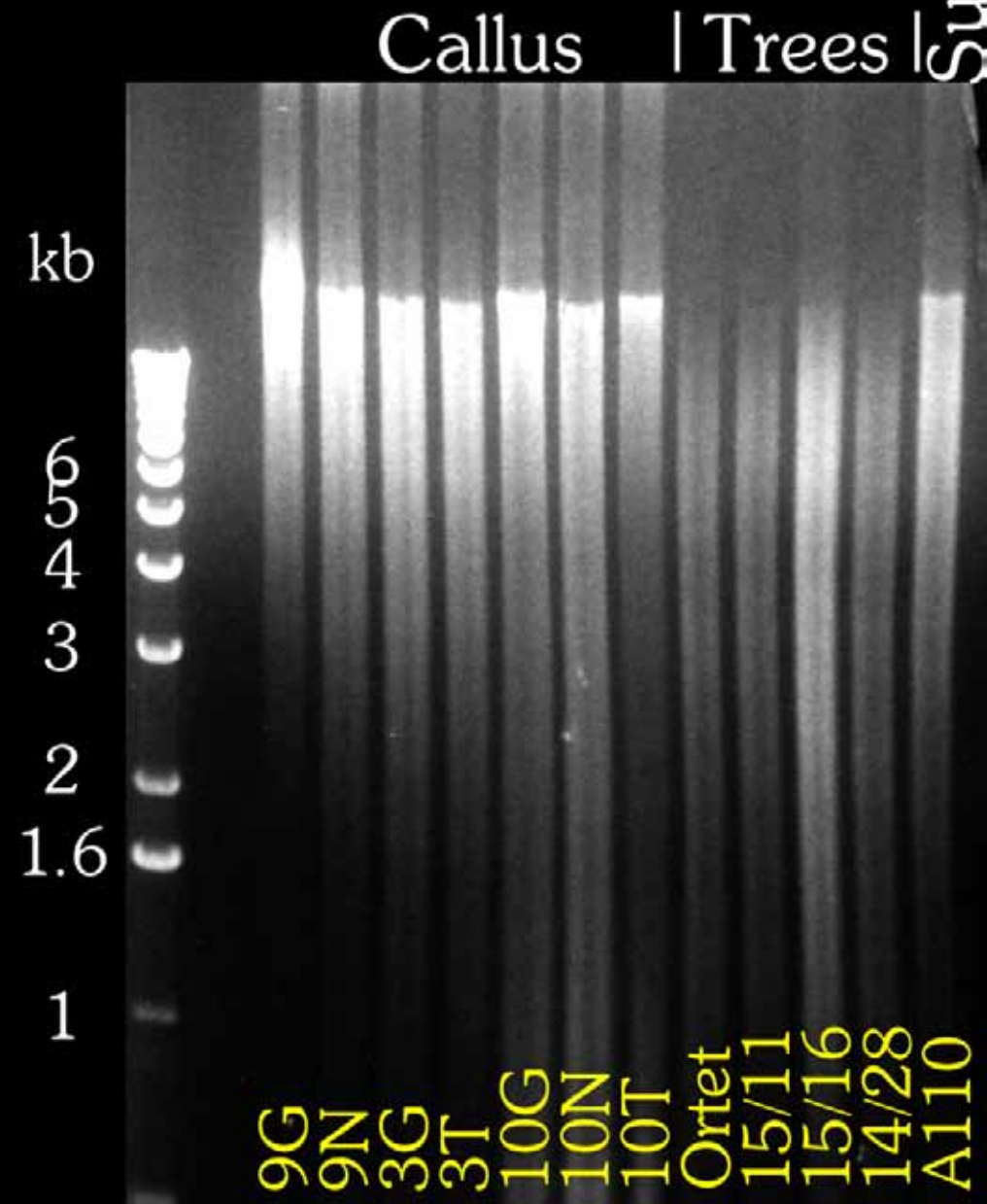
N typically 20 to 40 bases

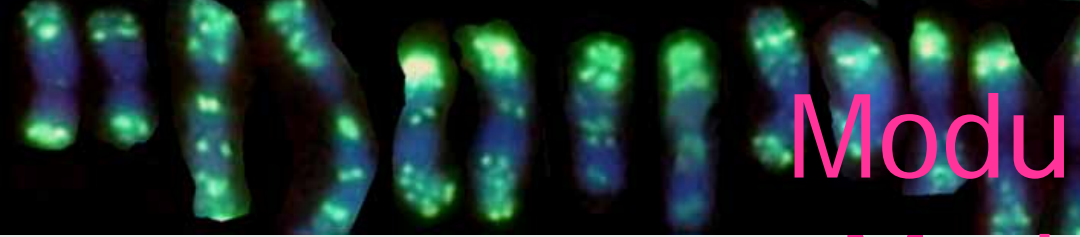


Modul Meth

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

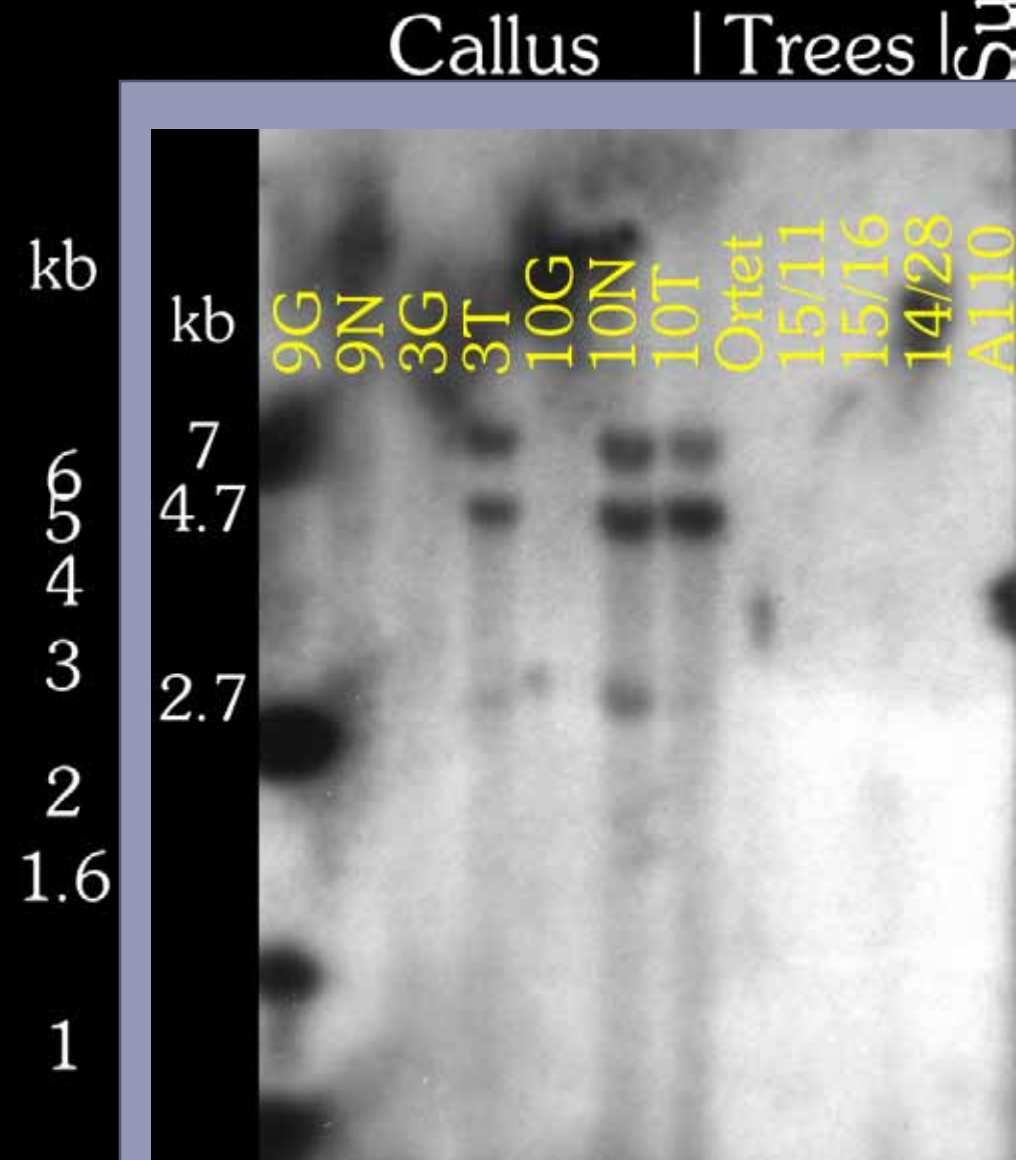
Kubis, Castilho, Vershinin, HH 2003





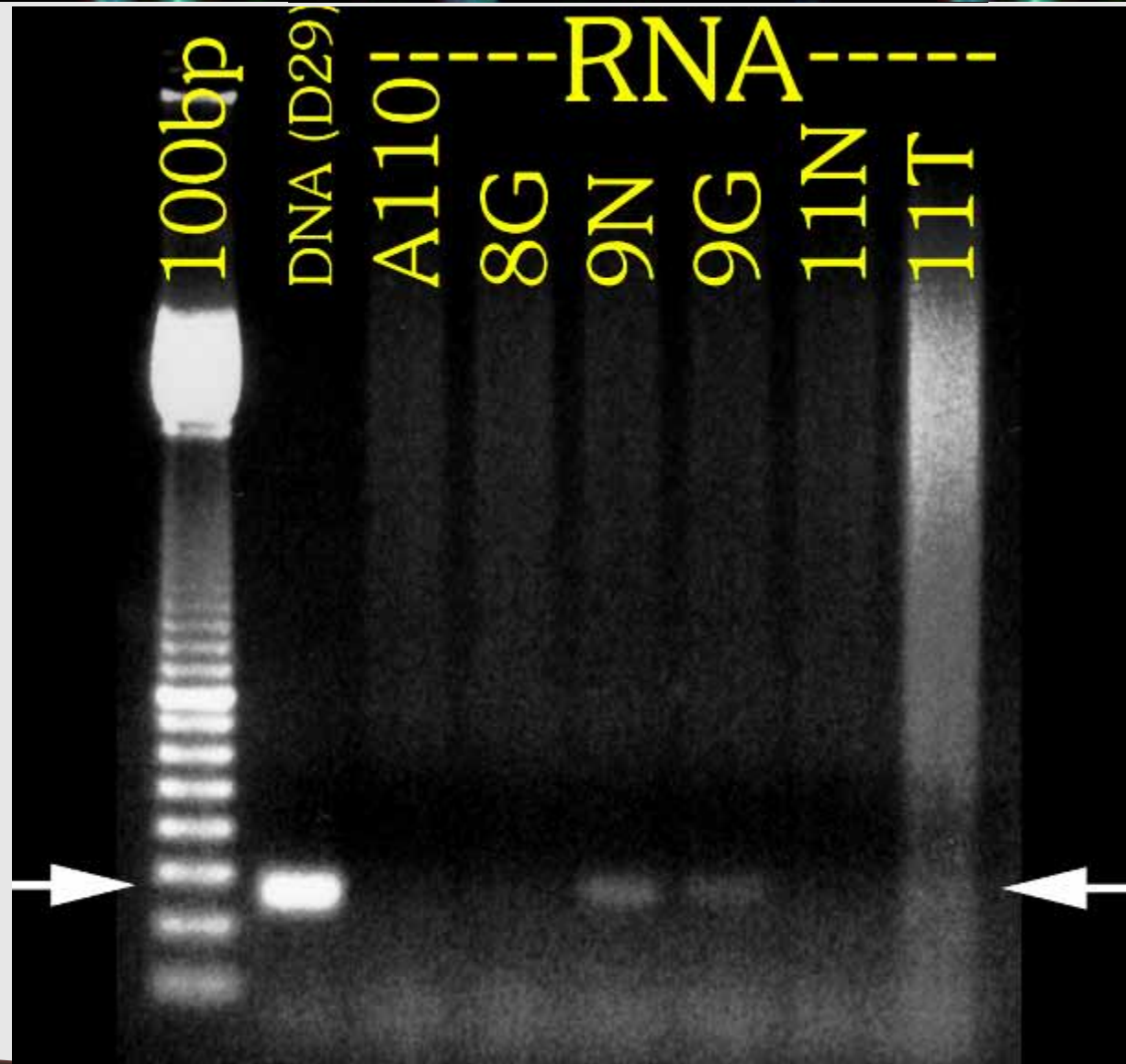
Modul Meth

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



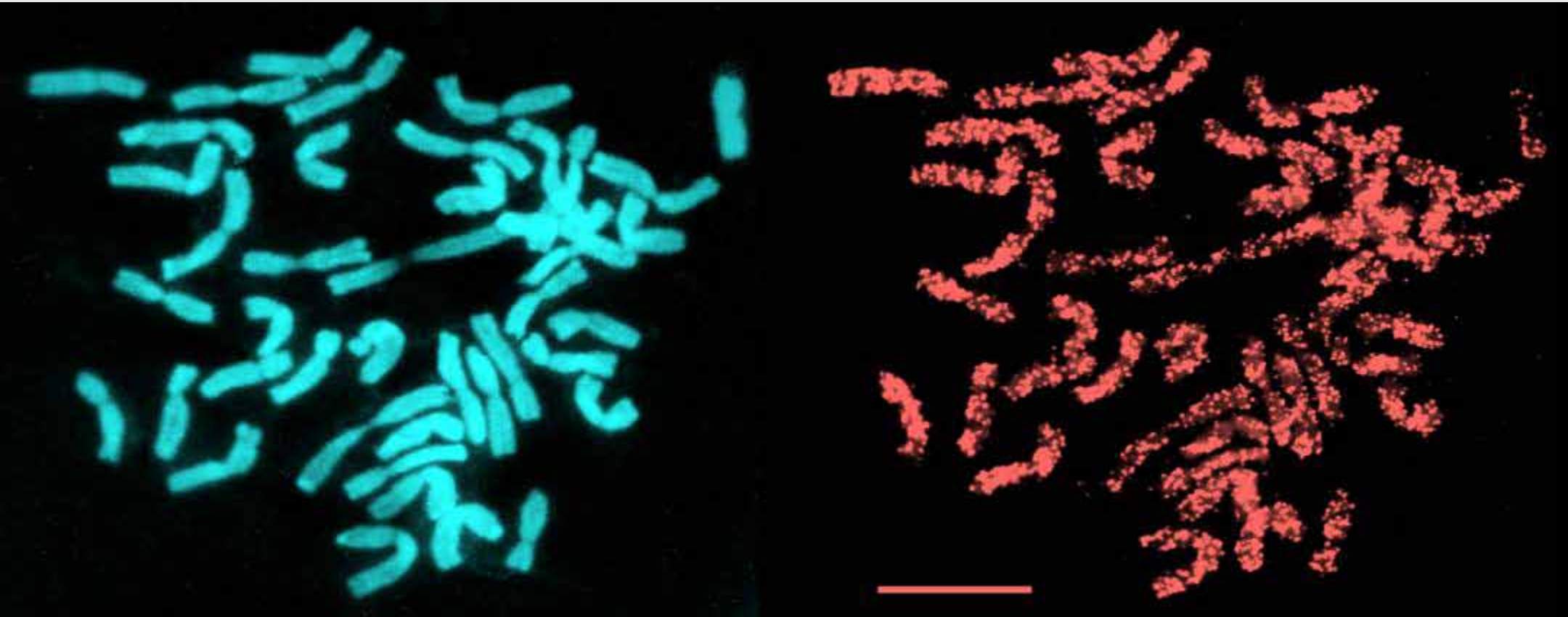
Transcriptional Activity

- ☛ *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

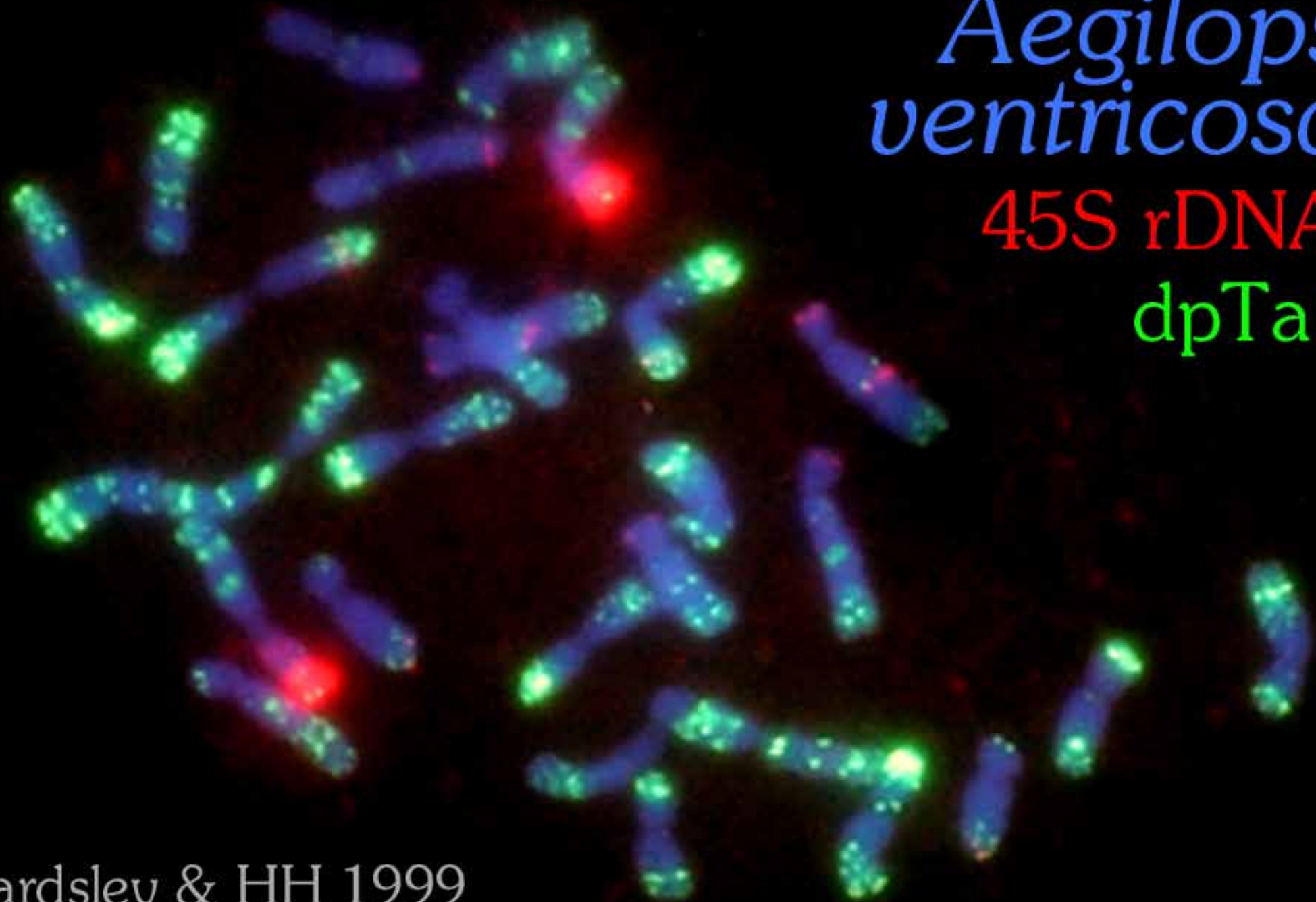
Histone modification



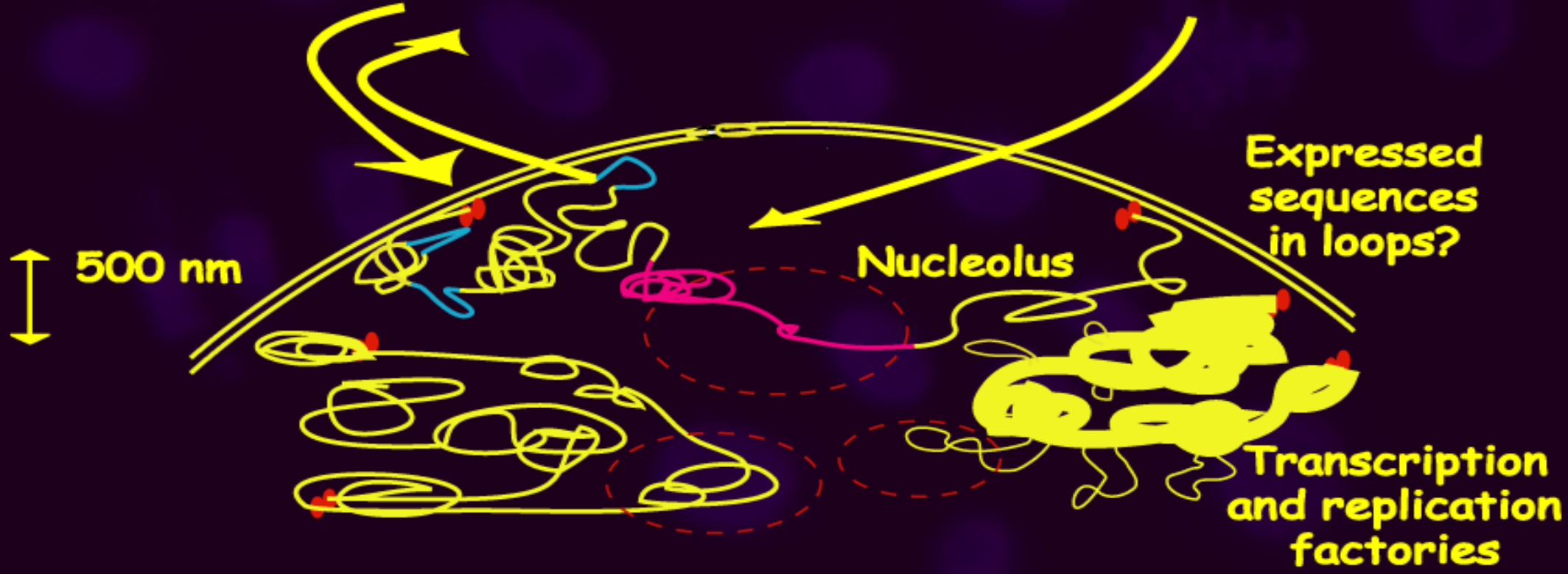
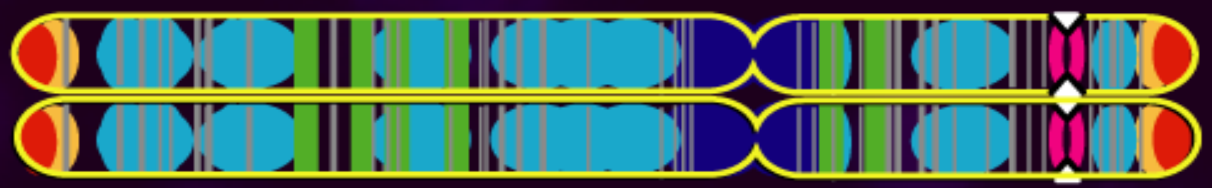
*Aegilops
ventricosa*

45S rDNA

dpTa1



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





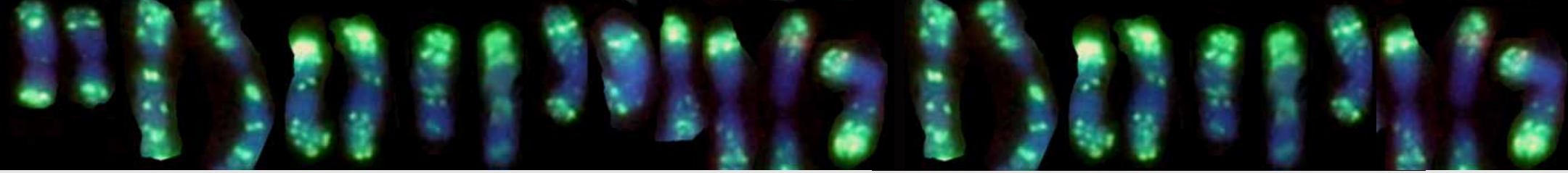
Super-Domestication: United Nations

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 worlds diversity

... needs for agriculture: not the only reasons for genomics
Moral imperative not to destroy that we have in the world



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

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

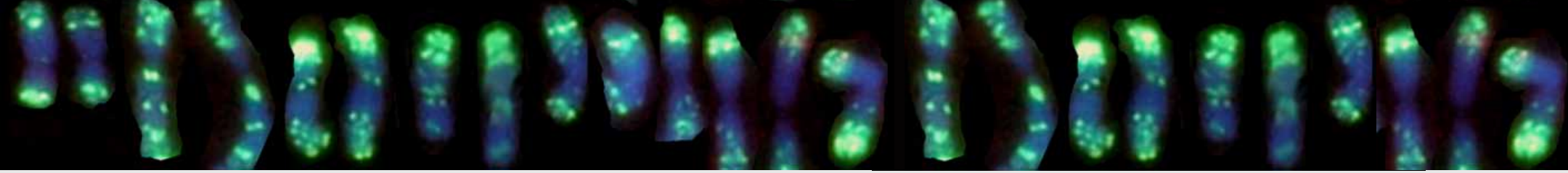
Future – Pollution and land use





Threats to sustainability: no different for 10,000 years

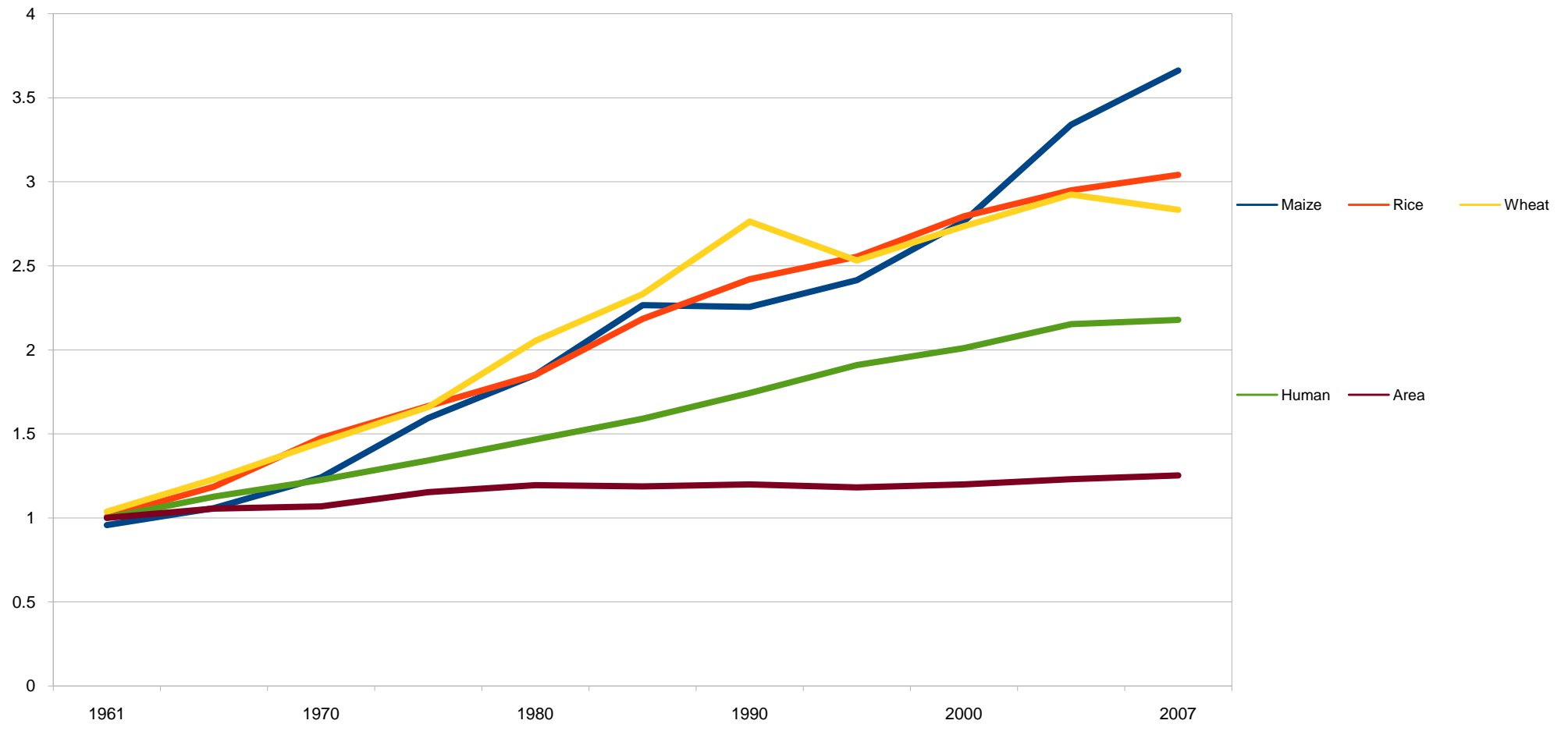
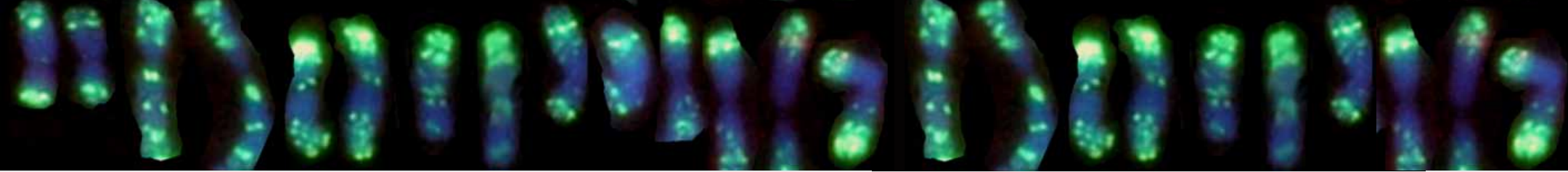
- ☞ Habitat destruction
- ☞ Climate change (abiotic stresses)
- ☞ Diseases (biotic stresses)
- ☞ Changes in what people want
- ☞ Blindness to what is happening
- ☞ Unwillingness to change



Genomics ...

- ☞ 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

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



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, guavas	33
Eggplants (aubergines)	32
Millet	32

FAO Statistics 2007

All plant crops with >30M tons annual production

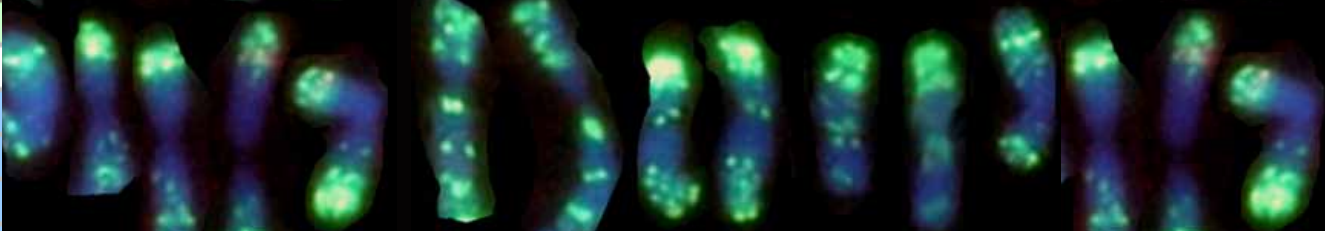
excluding sugar cane and 'other vegetables'

People: WHO

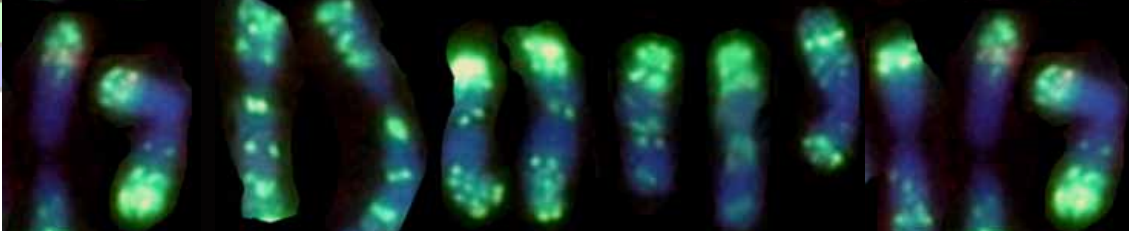
Calories are pretty important – 'let them eat micronutrients' is not the message

year (millions)

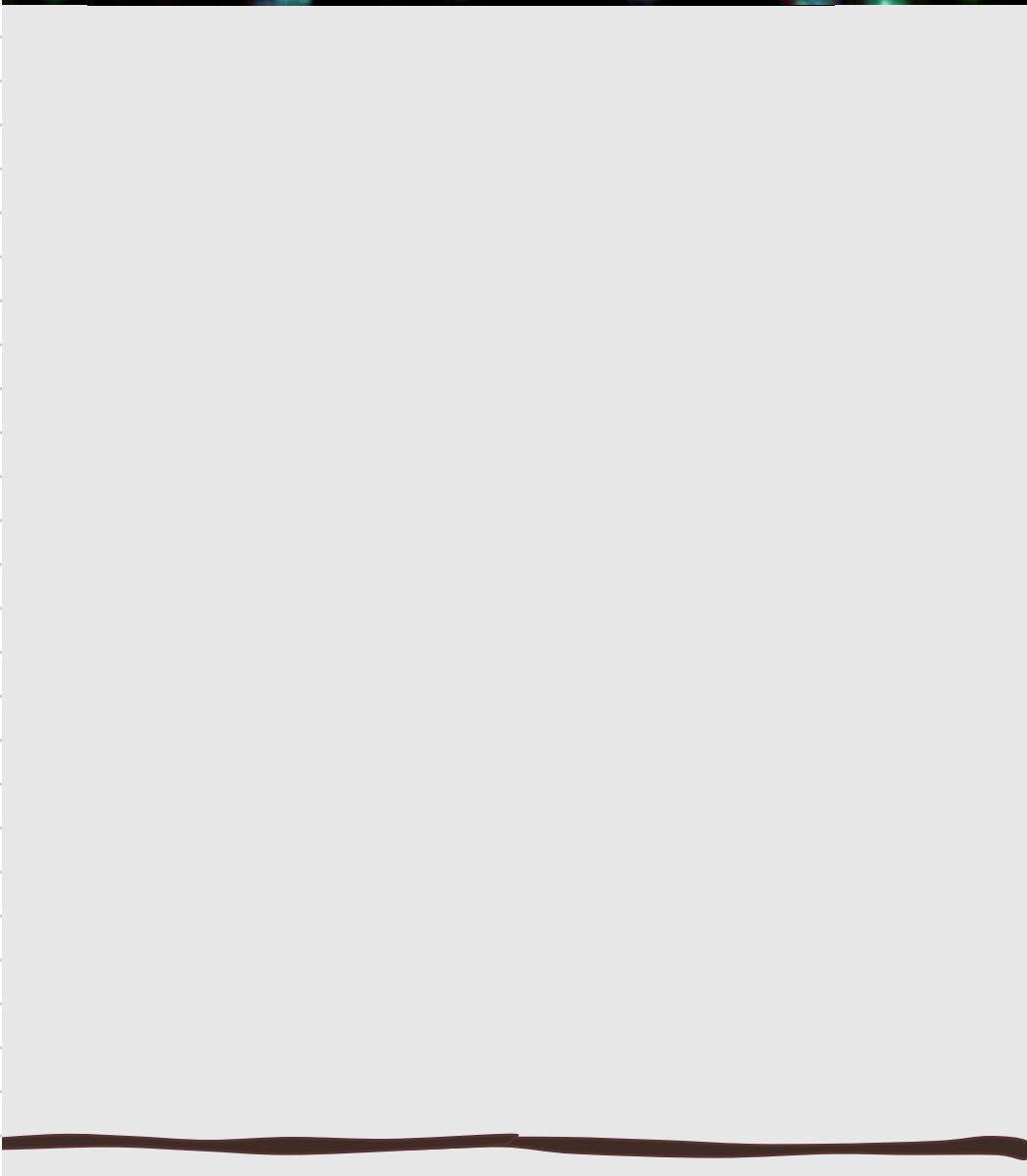
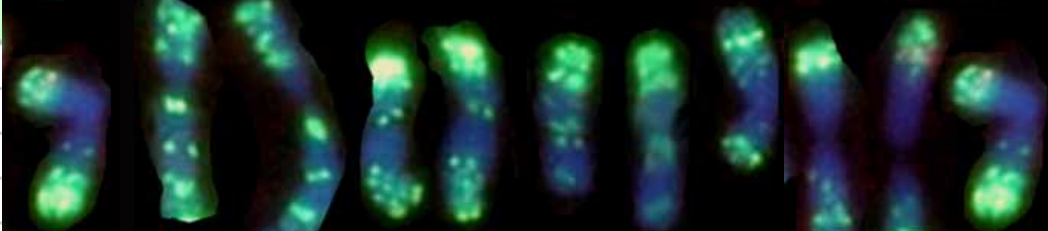
item	1961	2007
People	3,090	6,602
Maize	205	785
Rice, paddy	216	652
Wheat	222	607
Potatoes	271	322
Sugar beet	161	248
Cassava	71	228
Soybeans	27	216
Oil palm fruit	14	192
Barley	72	136
Sweet potatoes	98	126
Tomatoes	28	126
Watermelons	18	93
Bananas	21	81
Seed cotton	27	73
Cabbages and other brassicas	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)		
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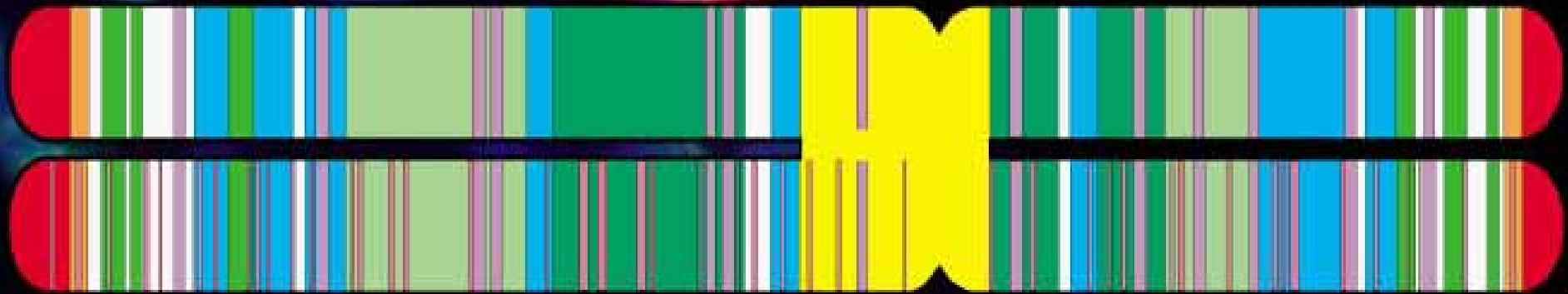




Plant Genome Evolution and Diversity

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

The Linear Chromosome

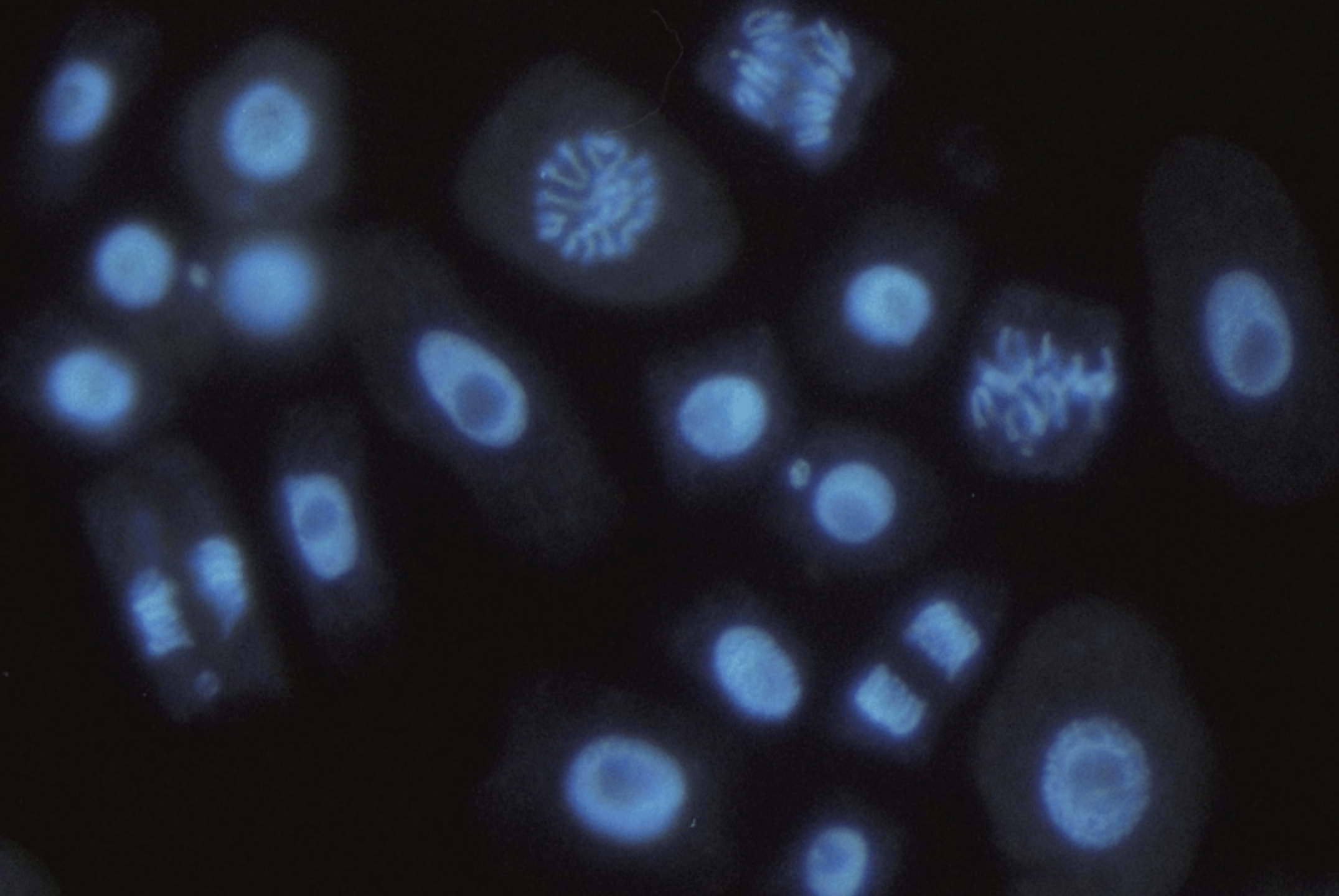


Tandem repeats

Retroelements
Simple sequence repeats

Terminal repeats

Genes



Chromosome Analysis and Molecular Cytogenetics

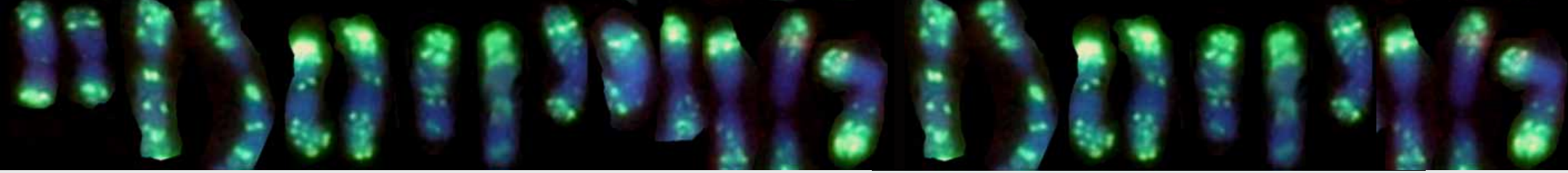
Pat Heslop-Harrison

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www.molecularcytogenetics.com

UserID/PW: 'visitor'



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