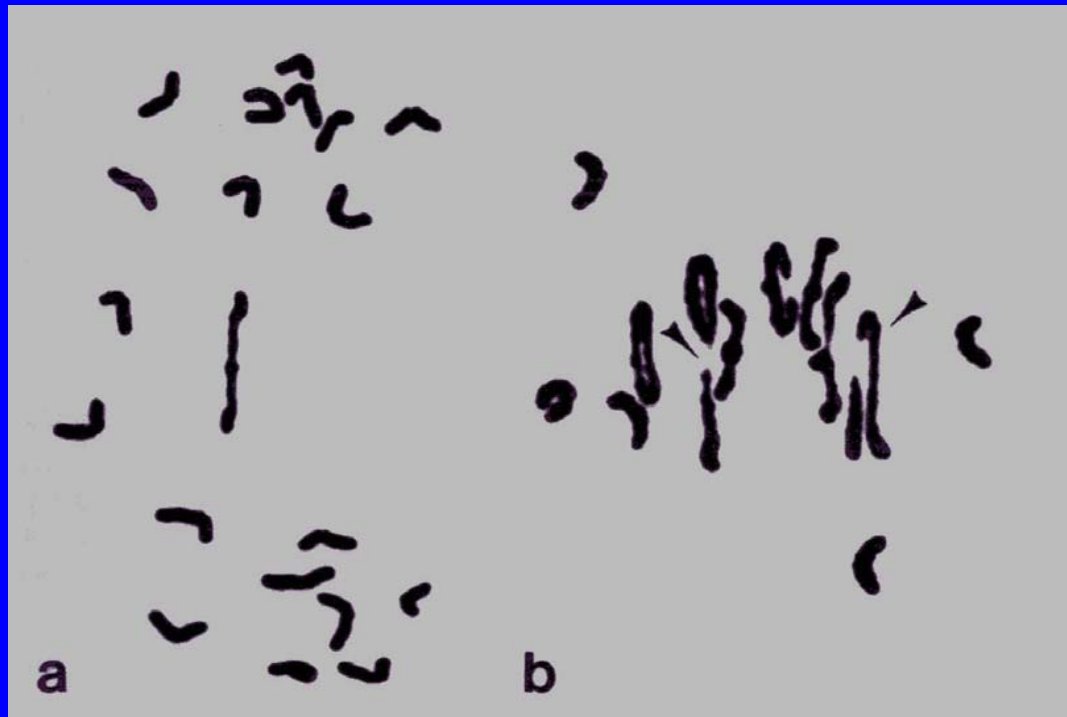


Wheat Chromosome Engineering and Breeding

Jianli Chen



Chromosome Engineering

- A process to transfer favorable alleles through **inter-specific hybridization** and interchange of chromatin using **aneupolids**

Aneuploids?

- Individuals having chromosome numbers other than an exact multiple of **the basic chromosome set**.
- **A basic chromosome set** contains all chromosomes in **a genome**.
- **A genome** is defined as **the basic chromosome set** that contains all the genetic information needed to produce an organism or an organelle, denoted by a x.

Review Concepts

n: gametic (haploid) chromosome number. $n = 3x = 21$ (wheat)

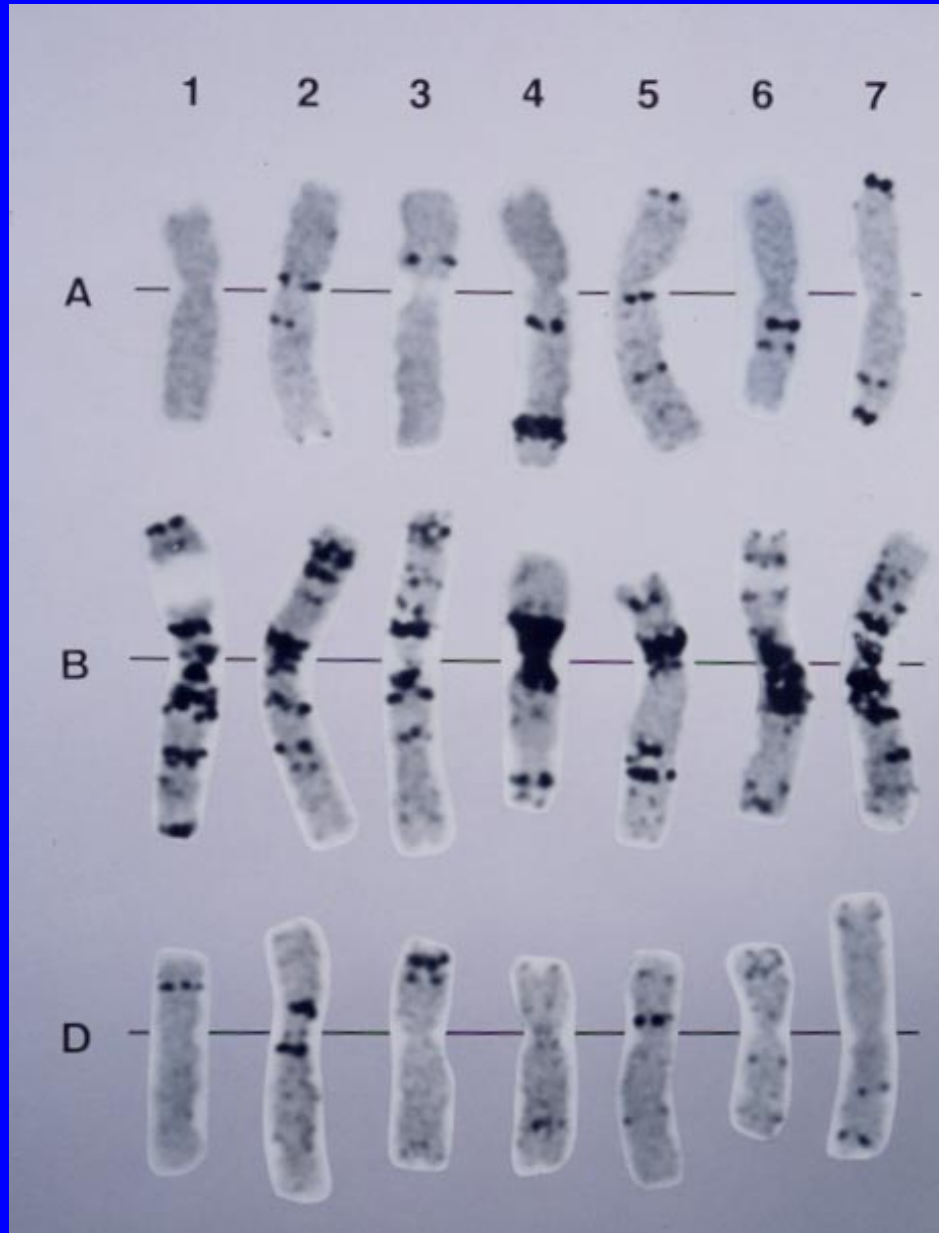
2n: disomic (somatic) chromosome number.

wheat: $2n = 6x = 42$; Barley: $2n = 2x = 14$

Soybean: $2n = 2x = 20$; Maize: $2n = 2x = 20$

Outline of current lecture

- **Types of aneuploids in common wheat**
- **Application of aneuploids in wheat genetic and mapping studies**
- **Application of aneuploids in wheat breeding**



Genetic features of common wheat

- Hexaploid ($2n = 6x = 42$) contains three closely related (homoeologous) genomes ($x = 7$), A, B, and D, which are derived from three diploid species (AA- *T. urartu*, BB- *Ae. Speltoides*, DD- *Ae. Squarrosa*)

‘Genetic Features’ con’t.

- Most of the genes have three homoeologous loci, which can functionally compensate for one another.
- Hexaploid with homoeologous genomes can tolerate loss or addition of chromosomes.
- Complete sets of aneuploids of Chinese Spring and other wheat varieties are available.

Available Wheat Aneuploid Series

- Monosomics (Sears, 1954)
- Nulli-tetrasomics (Sears, 1954).
- Nullisomics (Sears, 1954; Xue et al., 1990)
- Ditelosomics (Sears and Sears, 1978).
- Deletion stocks (Endo, 1978)

Monosomics ($2n-1$): a set of 21 (1AM)

- An individual **lacking of one chromosome** from the normal diploid (disomic) complement ($20\text{ II} + 1\text{ I}$ or 41).
- Plants look similar to the disomics and fertility is close to normal.
- Gametes are transmitted at a different rate through male and female.

Monosomic



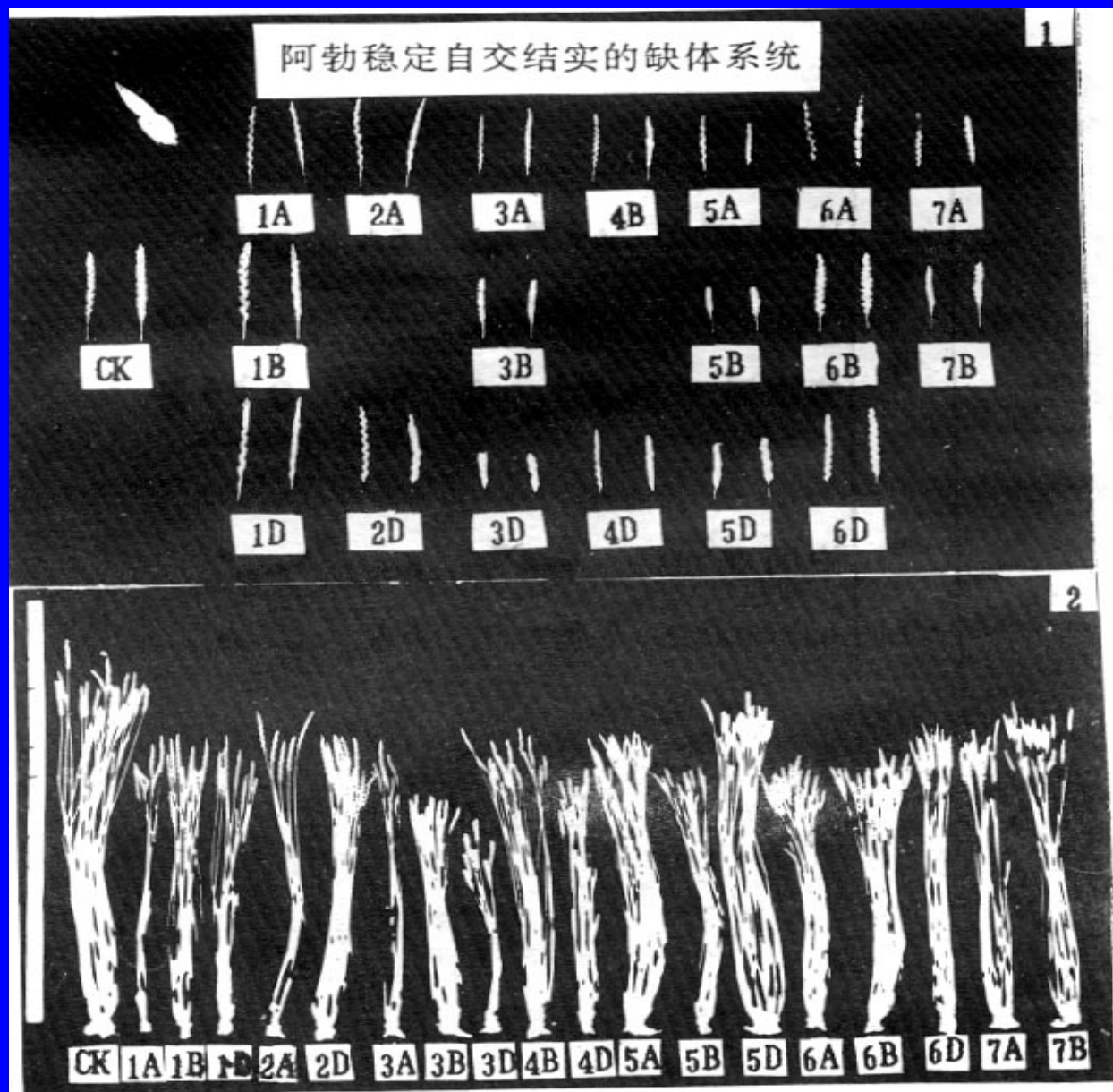
Expected Transmission of the monosomics in *Triticum aestivum*

Female Gamete	Male Gamete	
	n (21) 96%	n-1 (20) 4%
n (21) 25%	Disomic 2n (42) 24%	Monosomic 2n-1 (41) 1%
n-1 (20) 75%	Monosomic 2n-1 (41) 72 %	Nullisomic 2n-2 (40) 3%

Nullisomics ($2n-2$): a set of 21 (1AN)

- An individual **lacking of one pair of chromosomes** from the normal diploid complement ($20II$ or 40)
- Plants distinguishable by morphological features (vigor and size)
- Gametes transmitted via same rate in female and male

阿勃稳定自交结实的缺体系统



Nullisomic



Nulli-tetrasomics: a set of 42 (N1AT1D)

- An individual lacking of one pair of chromosomes but having a doubled pair of one chromosome from the homoeologous group (19 II + 1IV, or 42)
- Plants look similar to disomics and fertility is close to normal
- Gametes transmitted via similar rate in female and male

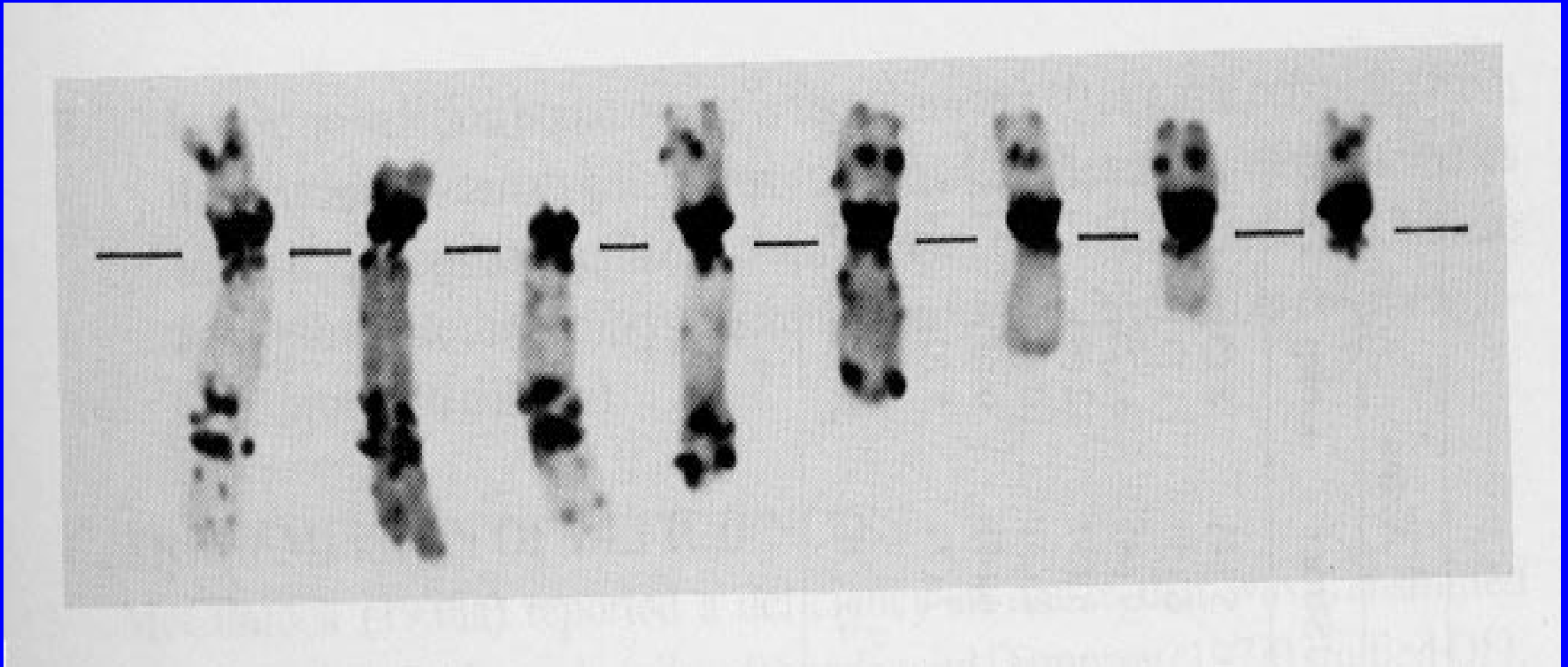
Ditelosomics: a set of 42 (1AS &1AL)

- An individual lacking of **a pair of chromosome arms** from the normal diploid complement
- Plants look similar to disomics and fertility is close to normal
- Gametes transmitted via same rate in female and male

Deletion stocks: unlimited

- An individual lacking **a segment of a chromosome** from the normal diploid complement
- fertility is based on the location of deletions
- Mainly used in physical mapping

5B Deletion stocks



(Endo, T.R.1990. Jpn. J. Genet. 65: 135-152)

Genetic Study

- Objective

Locate genes of interest:

on specific chromosome

on specific chromosome arm

on specific chromosome segment

Aneuploid Analysis

Practical considerations

- Availability of aneuploid stocks in your crop species
- Nature of genes: qualitative or quantitative; dominant or recessive

Locating Recessive Genes (1A is gene carrier)

Female		Male
(Awnless)		(Awn)
$20\text{II} + 1\text{I}_{1A}$	X	$20\text{II} + 1\text{II}_{1aa}$
↓		
F ₁	75%	$20\text{II} + 1\text{I}_{1a}$
	25%	$20\text{II} + 1\text{II}_{1Aa}$
3(Awn and monosomic)		
1(Awnless and disomic)		

Female		Male
(Awnless)		(Awn)
$19\text{II} + 1\text{II}_{1AA} + 1\text{I}_{1b}$	X	$20\text{II} + 1\text{II}_{1aa}$
↓		
F ₁	75%	$19\text{II} + 1\text{II}_{1Aa} + 1\text{I}_{1b}$
	25%	$19\text{II} + 1\text{II}_{1Aa} + 1\text{II}_{1bb}$

All plants are awnless

Monosomic analysis

	Male gamete
Female gamete	n (21)
n (21 chr., 25%)	Disomic ($2n = 42$, 25%)
n-1 (20 chr., 75%)	Monosomic ($2n-1 = 41$, 75%)

Locating Dominant Genes

	Female		Male
	(S)		(R)
	$20\text{II} + 1\text{I}_{1a}$	X	$20\text{II} + 1\text{II}_{1AA}$
	↓		
F ₁	75% $20\text{II} + 1\text{I}_{1A}$ 25% $20\text{II} + 1\text{II}_{1Aa}$		
	↓		
F ₂	4% S -- nullisomic 73% R -- monosomic 24% R -- disomic		

	Female		Male
	(S)		(R)
	$19\text{II} + 1\text{II}_{1aa} + 1\text{I}_{1b}$	X	$19\text{II} + 1\text{II}_{1AA} + 1\text{II}_{1bb}$
	↓		
F ₁	75% $19\text{II} + 1\text{II}_{1Aa} + 1\text{I}_{1b}$ 25% $19\text{II} + 1\text{II}_{1Aa} + 1\text{II}_{1bb}$		
	↓		
F ₂	75% R to 25% S		

Expected Transmission of the monosomics in *Triticum aestivum*

Female Gamete	Male Gamete	
	n (21) 96%	n-1 (20) 4%
n (21) 25%	Disomic 2n (42) 24%	Monosomic 2n-1 (41) 1%
n-1 (20) 75%	Monosomic 2n-1 (41) 72 %	Nullisomic 2n-2 (40) 3%

Locating Dominant Genes

(Nullisomic Analysis)

	Female		Male
	(S)		(R)
	20II	X	20 II + 1II _{1AA}
		↓	
F ₁	20 II + 1II_{1A}		
		↓	
F ₂	4% S -- nullisomic 73% R --monosomi 24% R -- disomic		

	Female		Male
	(S)		(R)
	19II + 1II _{1aa}	X	19II + 1II _{1AA} + 1II _{1B}
		↓	
F ₁	19II + 1II_{1Aa} + 1II_{1B}		
		↓	
F ₂	75% R to 25% S		

Genome Mapping

Objective

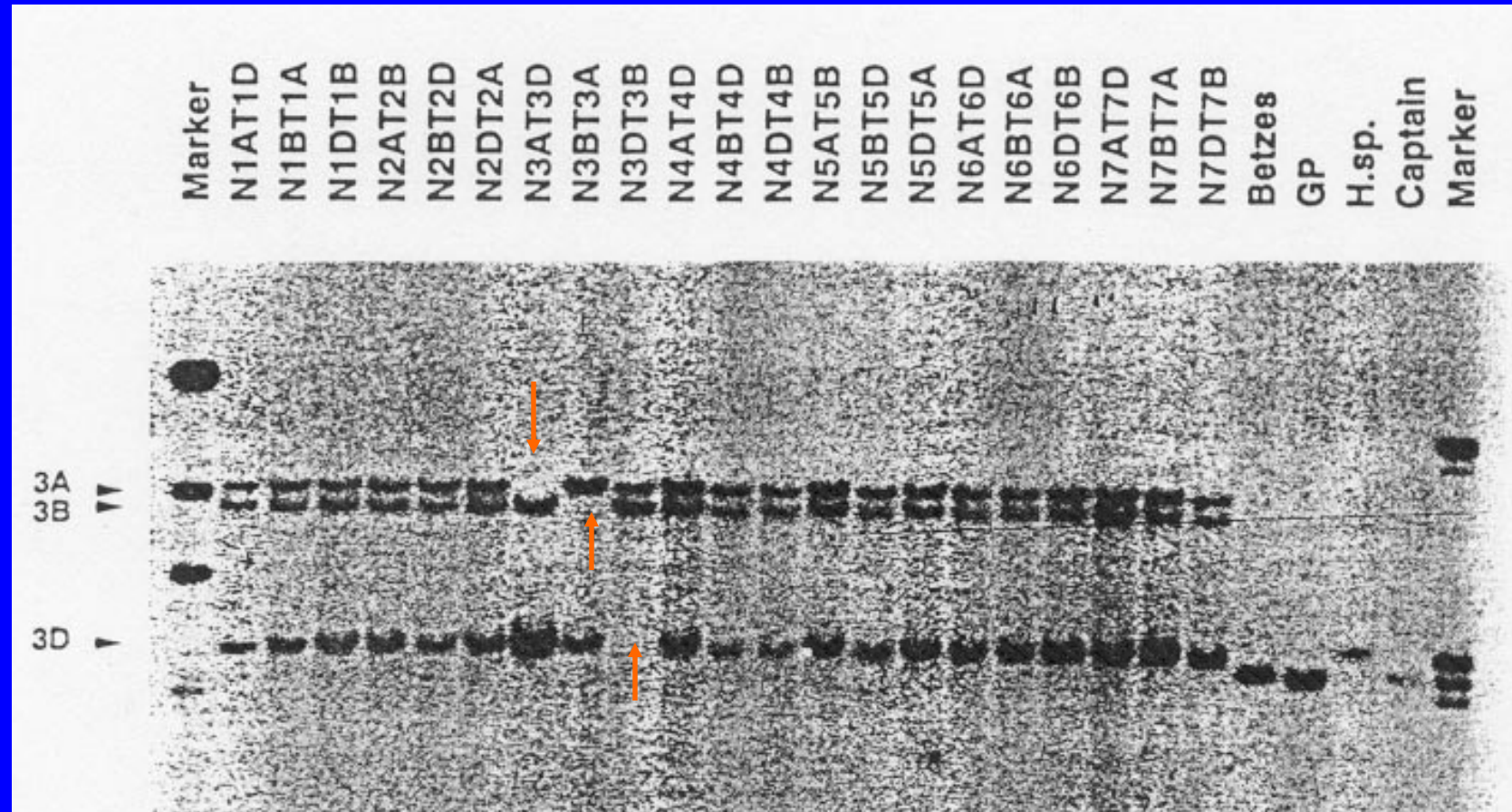
Map genes of interest or gene tightly linked
markers :

- on specific chromosome

- on specific chromosome arm

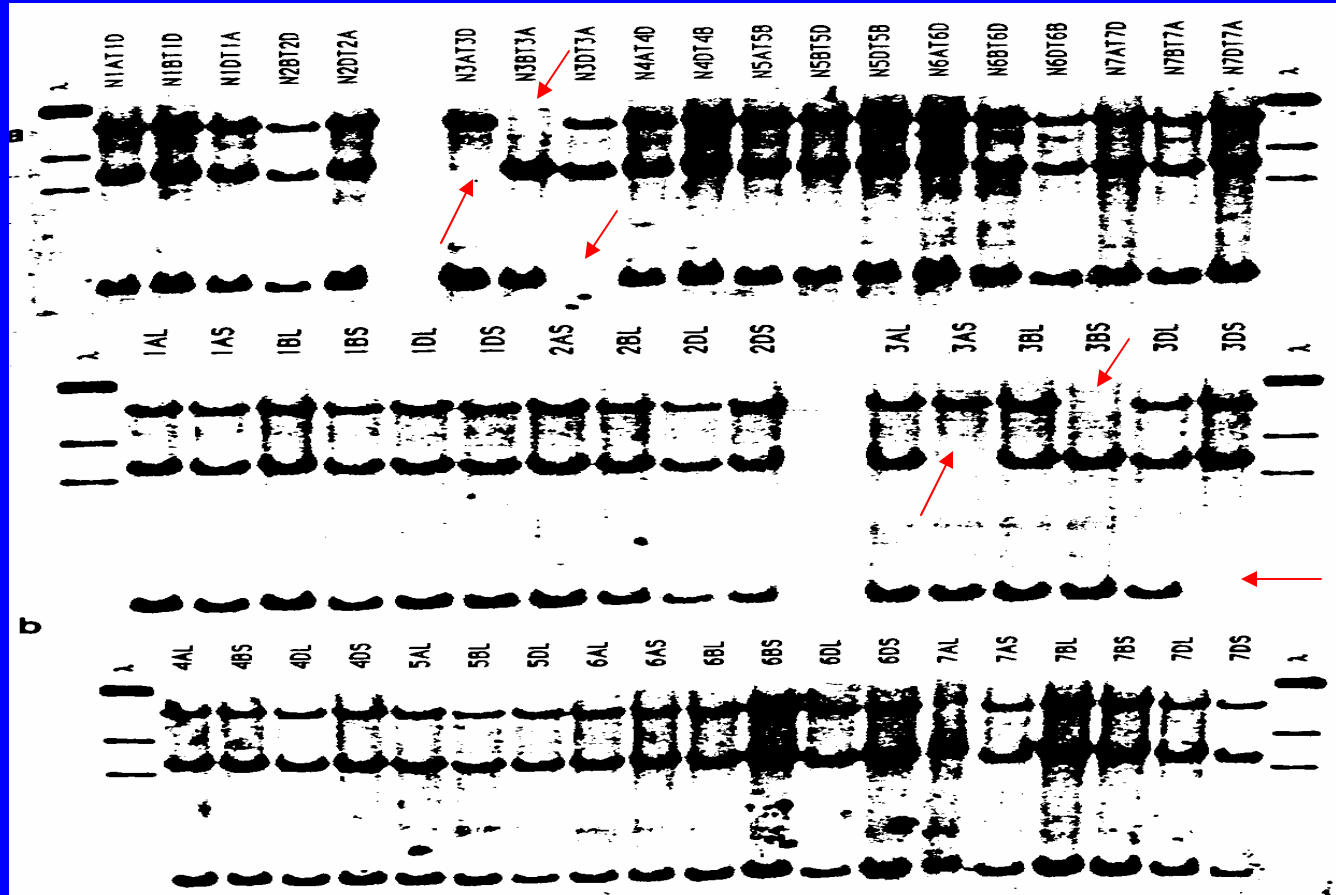
- on specific chromosome segment

Identification of the chromosomal location of a single copy probe PSR78 in hexaploid wheat



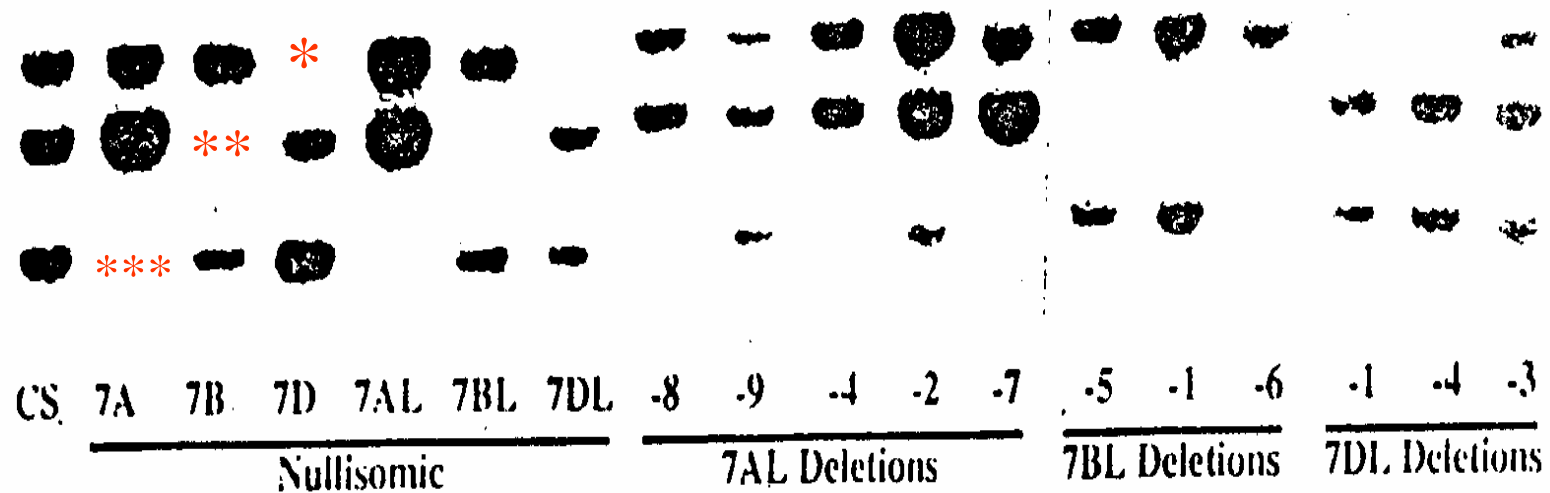
(Devos KM and Gale MD. 1993. Theme:93-99)

Chromosomal location of a RFLP probe BCD1127 in hexaploid wheat



(J.A. Anderson et al. 1992. TAG 83:1035 - 1043)

Localization of Gene-linked Markers on Chromosome Segment



Variety Improvement: interspecific hybridization

- Introgression of useful agronomic traits (Lr, Pm) from alien species to common wheat;
- Issues on interspecific crosses
 - sterile or partially sterile in F1
 - effective way to tag introduced chromosome or chromosome segments
 - Chromosome banding, genomic in situ hybridization, and molecular marker assisted selection

Procedures for transferring useful genes from alien species to common wheat

- Screening of donor populations
- Producing hybrids
- Chromosome doubling/backcrossing
- Production and identification of alien addition/substitution lines
- Induction of recombination
- Screening/stabilizing recombinants/translocations
- Gene tagging
- Newer technologies

Sources of useful agronomic traits in alien species

Disease resistance

- Powdery mildew – *Ae. Markgrafii*, *Ae. Comosa*, *D. villosum*, *T. spelta*, *T. diccoides*, *T. macha*, *Ae. kotschii*
- Leaf rust – *Ae. Caudata*, *T. monococcum*, *T. tauschii*, *Th. Distinchum*
- Stem rust – *T. diccoides*, 2R, *T. tauschii*
- Yellow rust – *T. spelta*, synthetic hexaploids
- Fusarium head blight – *H. chilense*, *L. elongatum*, *Roegneria*, *S. cereale*
- Karnal bunt – *triticales* (4R, 6R), *T. monococcum*

‘Procedures’ con’t.

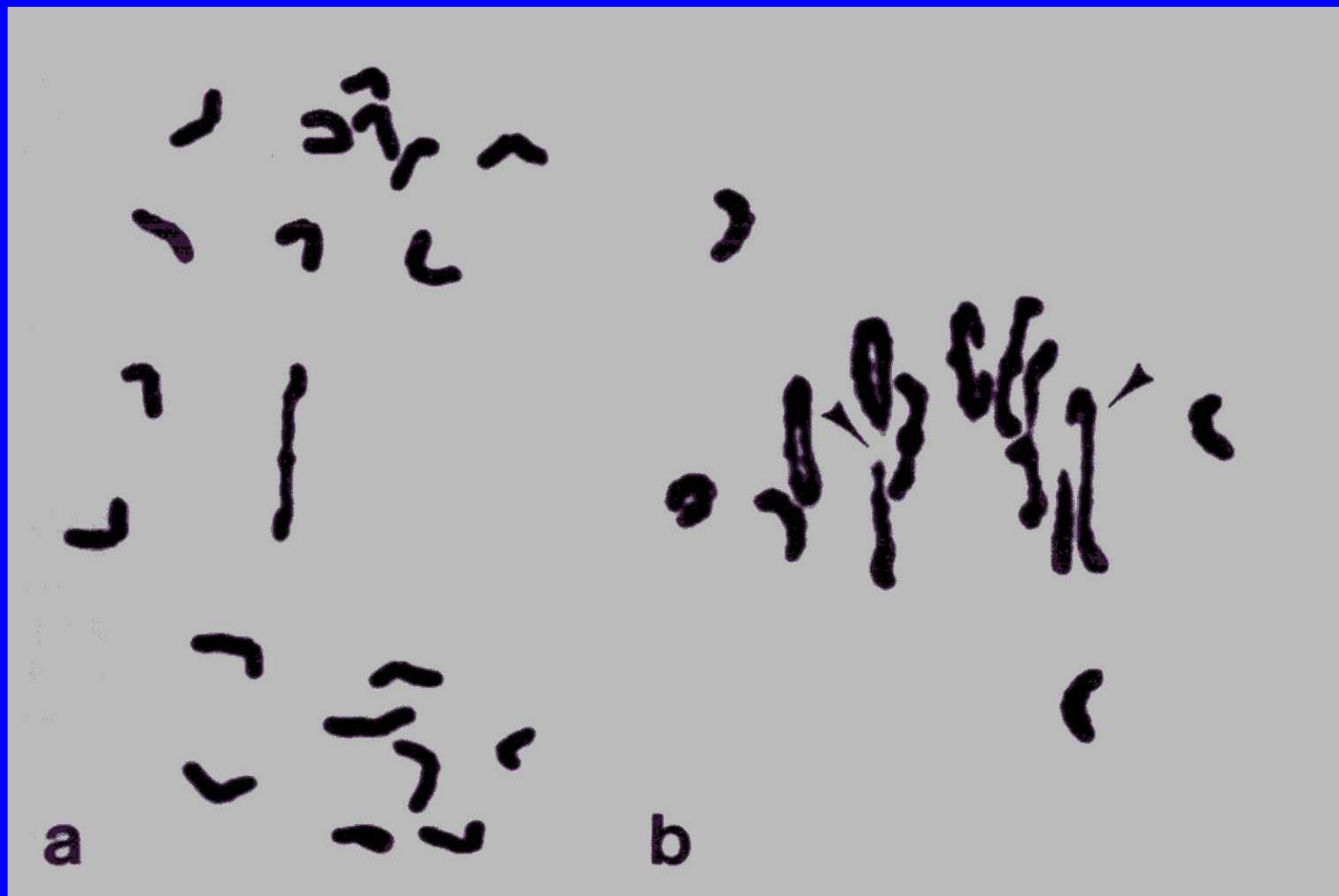
- Wild hybridization (wheat x Rye – triticales)
- Production and identification of alien addition or substitution lines
 - Aneuploids x wild species followed by doubling and backcrossing
- Production and identification of translocation lines

Method of Translocation Induction

- Tissue culture (BYDV) (Bank et al., 1995)
- Radiation (Lukaszewski, 1995)
- **The 5B system – induction of homeologous pairing (Sears, 1977)**

The 5-B system in wheat

- The 5B system, a Ph gene (homoeologous pairing suppressor) is a genetic control which restricts chromosome pairing to homologs;
- When Ph is **removed**, or its activity is **suppressed**, not only do homoeologous chromosomes pair but they also pair with the chromosome of related species and genera, making alien gene transfer possible (Sears, 1975, 1976).

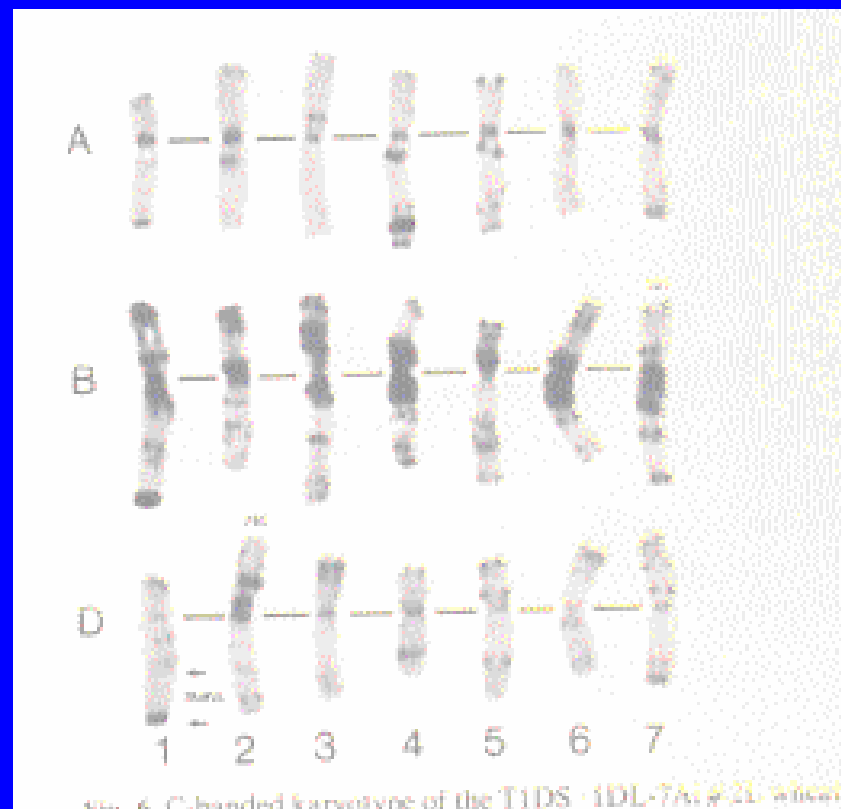
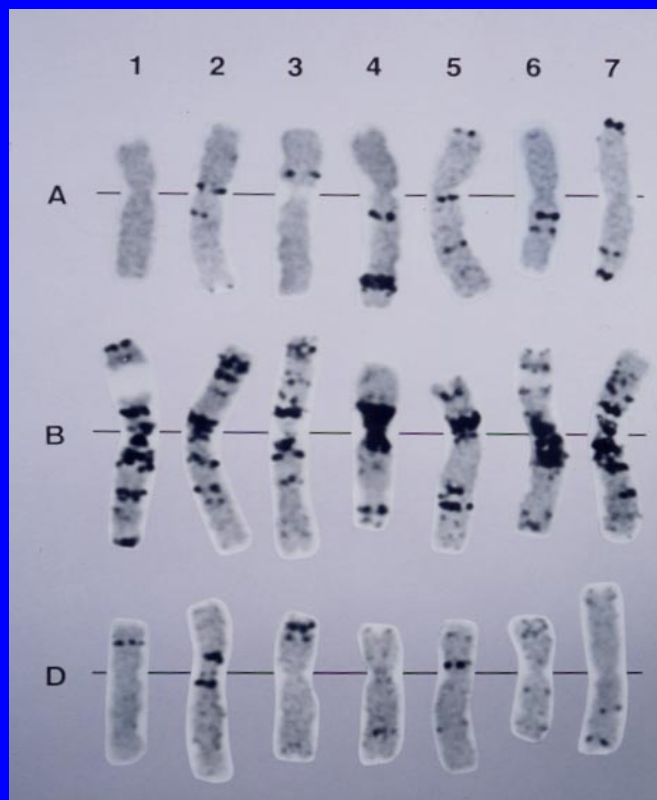


Chromosome paring in polyhaploids of bread wheat ($2n = 21$) with (a) and without (b) *Ph* gene (from Jauhar et al., 1991)

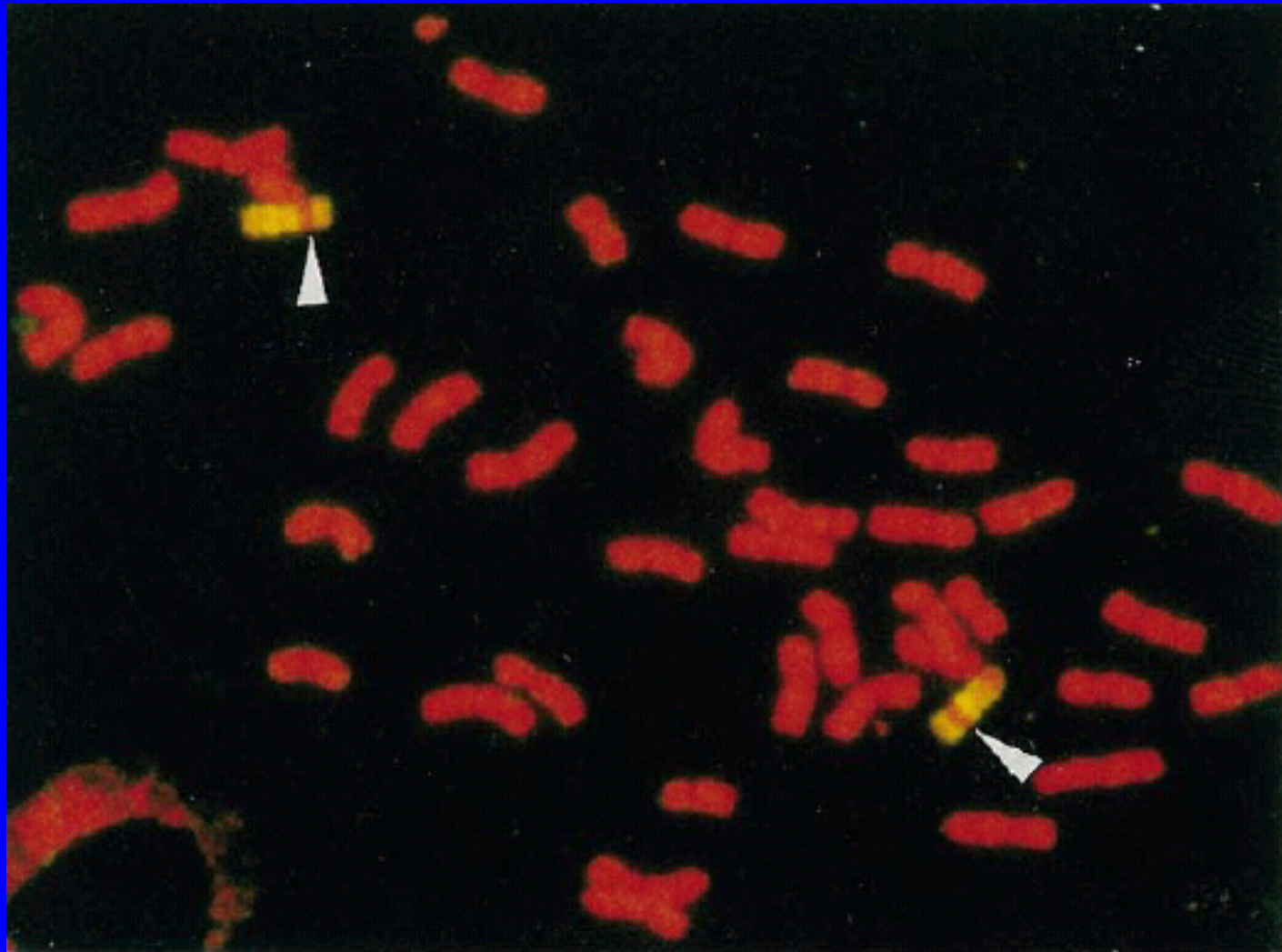
Procedures for inducing homoeologous pairing

1. Monosomic or Nullisomic 5B x Alien species, F_1 x adapted lines or variety;
2. Monosomic or Nullisomic 5B x Alien addition or substitution lines, F_1 x adapted lines or variety.

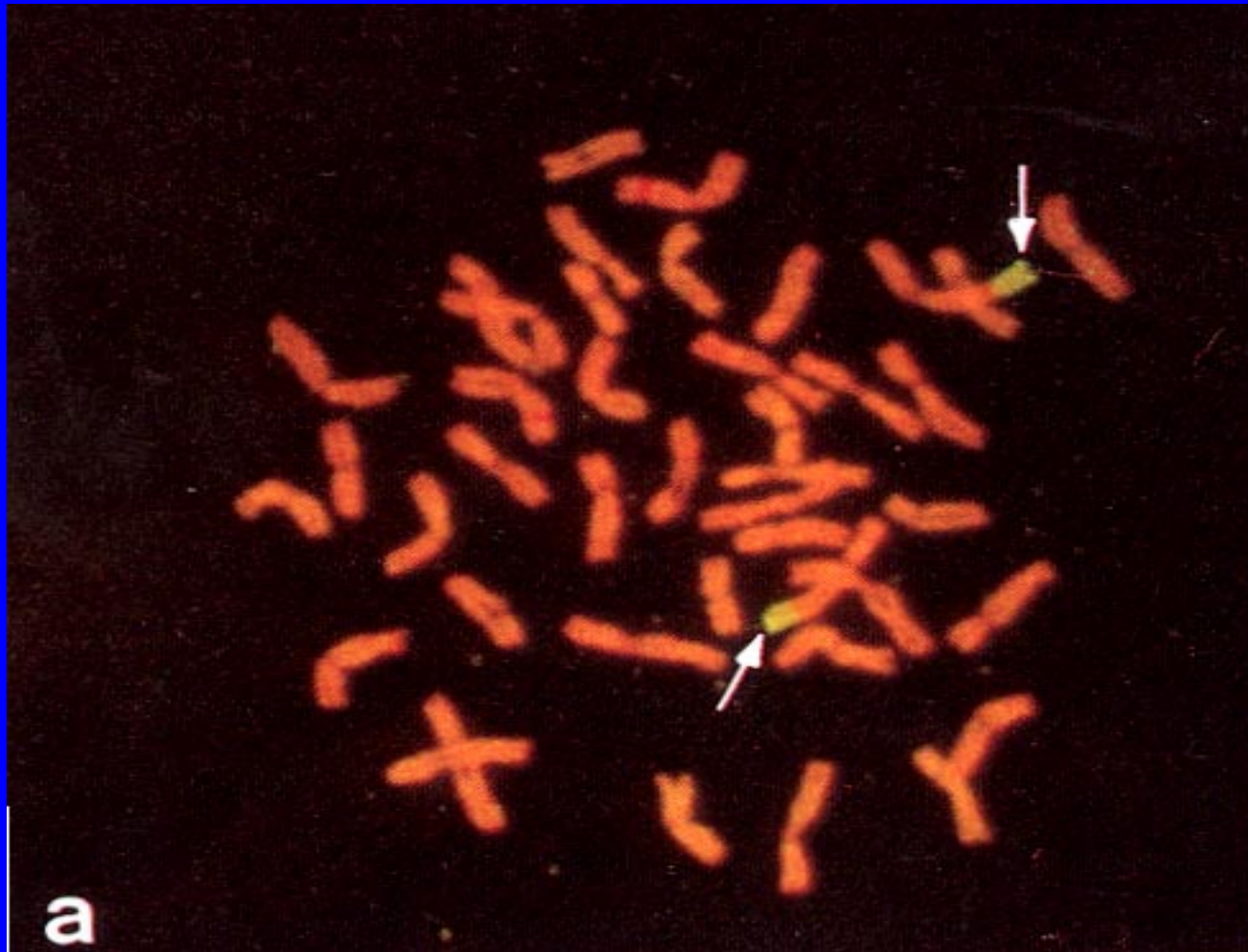
Identification wheat-alien translocation lines



Identification wheat-alien substitution lines



Identification wheat-alien translocation lines



RAPD analysis in 10 individuals of two addition lines from JC1050

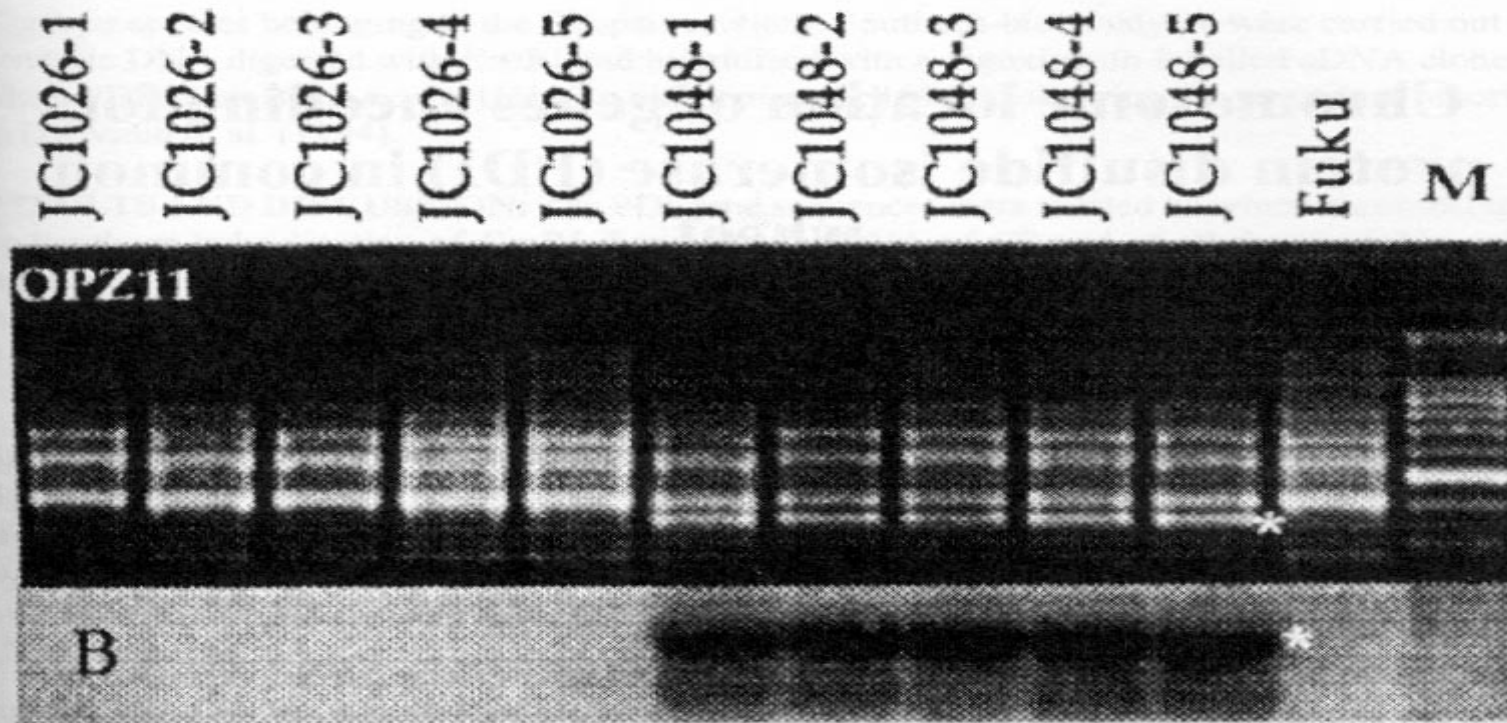


Figure 3 RAPD analysis in 10 individuals of two addition lines from JC1050. RAPD was performed by primer OPZ11(C), and the product was hybridized with OPZ11-350 (C1).

Conclusion

- Aneuploids are unique in hexaploid wheat.
- Genome analysis in wheat has served as a model in other plant systems, and has made tremendous advances.
- Chromosome engineering will continue to make contributions to wheat improvement as new techniques become available.