Chromosome Research in the *Festuca-Lolium* Complex

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Institute of Experimental Botany of the AS CR, v. v. i.

Olomouc Research Centre

- Two laboratories:

  - **Laboratory of Molecular Cytogenetics and Cytometry (Dr. J. Doležel)**

  - Laboratory of Plant Cytoskeleton and Cell Cycle (Dr. P. Binarová)

- 38 employees

  - 13 PhD.

- Collaboration with Faculty of Science (Palacky University in Olomouc) - BSc.. MSc. a PhD. students
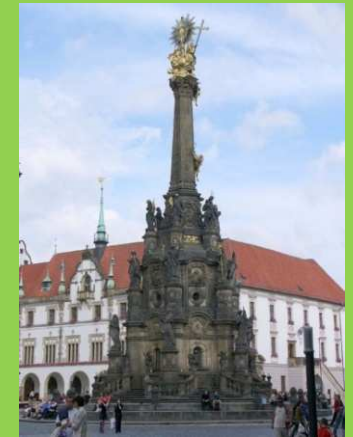
Research Topics

- Structure and evolution of plant genomes

- Our experimental subjects:

  - Bananas and Plantains (*Musa* spp.)

  - Cereals (wheat, barley, rye, maize)

  - Forage and Turf Grasses

Outline

Festuca-Lolium Complex Chromosome Research

- genomic composition of hybrids

- karyotyping

- genome size

- chromosome pairing/recombination study

- DArTFest array

Research Potential in Buffalograss and Blue Grama

Festuca - Lolium Complex

Lolium multiflorum Lam. (2n=2x=14; 2n=4x=28)
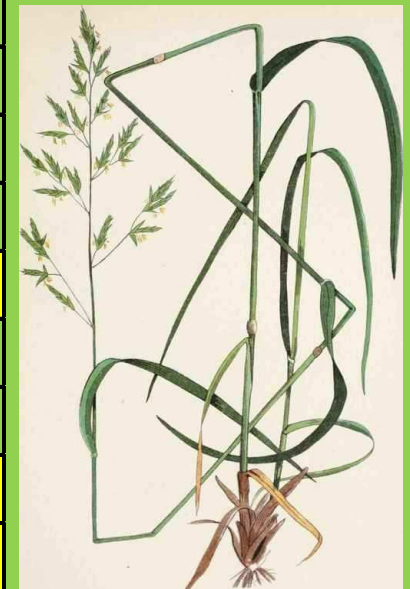*Lolium perenne L.* (2x=2x=14; 2n=4x=28)
Festuca pratensis Huds. (2x=2x=14; 2n=4x=28)
Festuca glaucescens Boiss. (2n=4x=28)
Festuca arundinacea Schreb. (2n=6x=42)

Some agronomic charactetistics	Lm	Lp	Fp	Fa
Rapid establishment from seed	+	+/-	-	-
Seed production	+	+/-	-	-
Palatability	++	+/-	+	-
Persistence	-	+	+	+
Resistance to treading	-	+	-	-
Winter hardiness	-	+/-	++	+
Drought tolerance	-	-	+	++
Sustained productivity	-	-	+	+

Festuca x *Lolium* Hybrids (Festulolium)

- Appear in nature (UK, France), but sterile

- From 1960´s, several breeding programs (Kenhy!)

- Combine complementary agronomic attributes of both genera

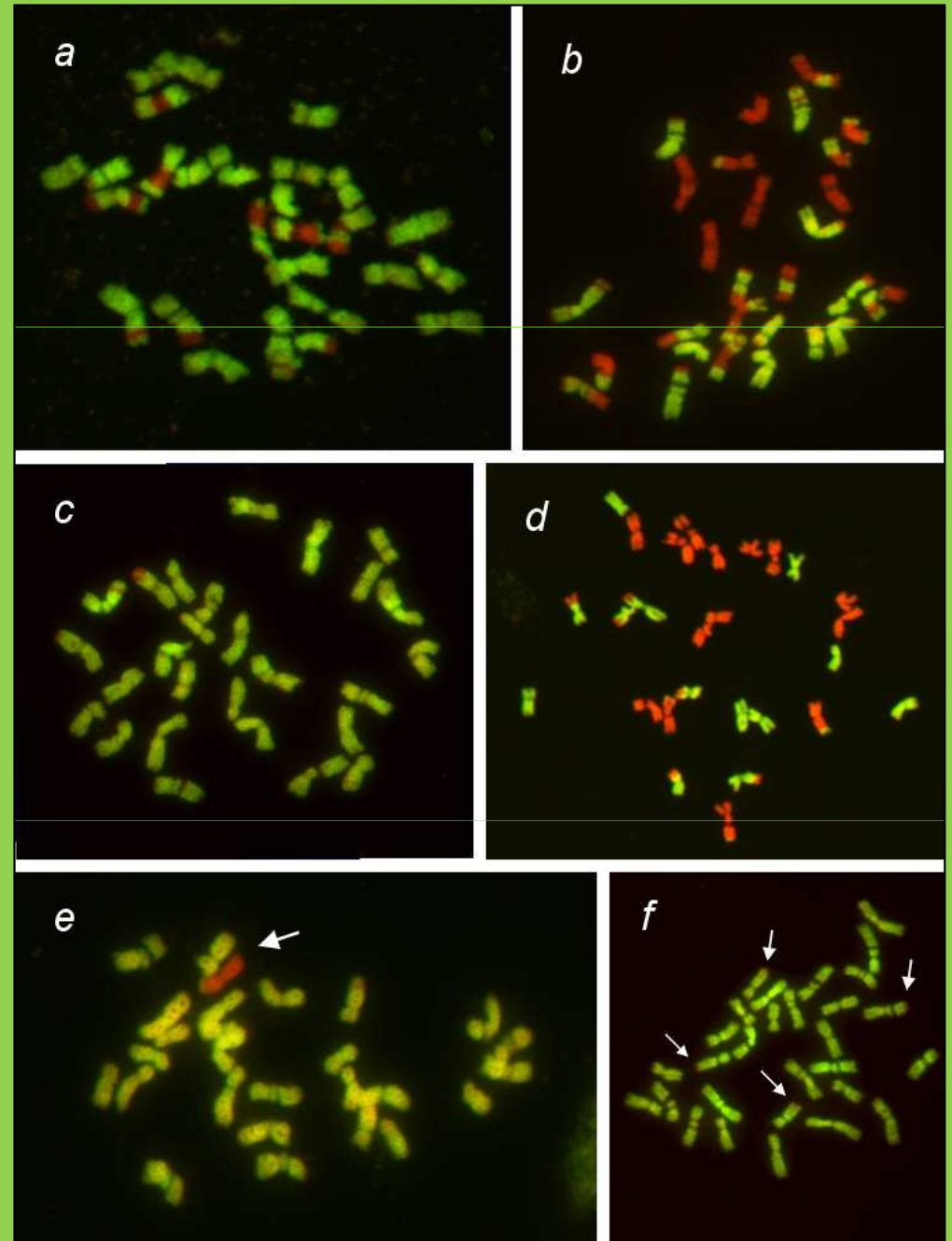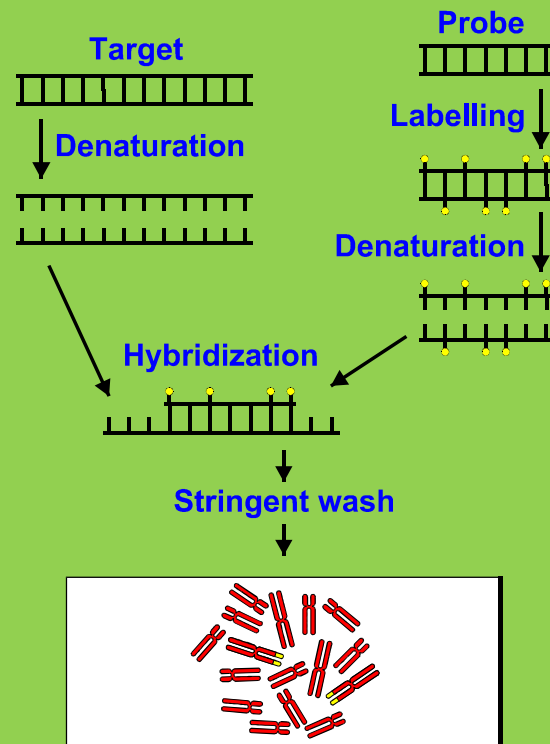- Over 40 cultivars (forage and turf) registered and released

Genomic constitution of hybrid cultivars
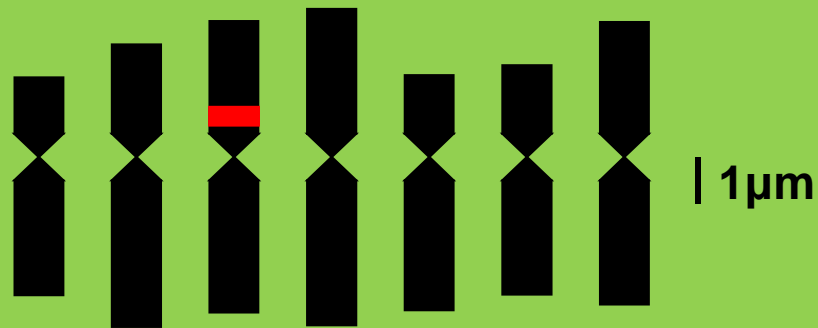
Number of Festulolium cultivars: ~42

Screened here: 25

Fluorescent *in situ* hybridization (FISH)
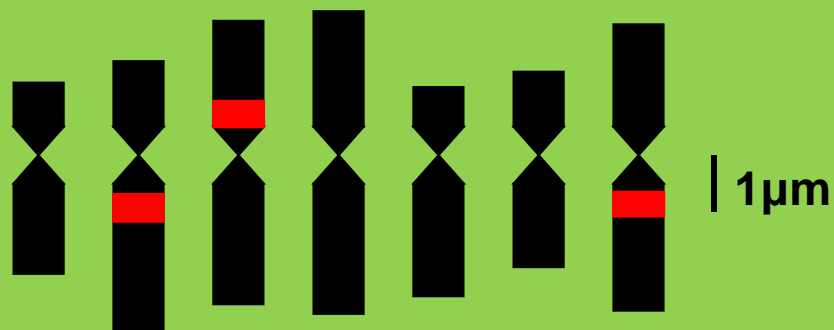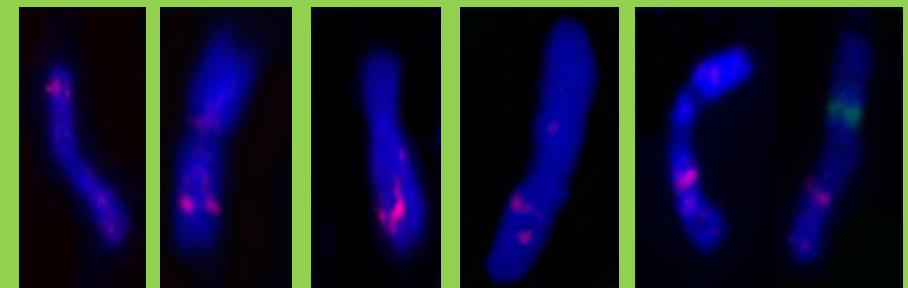**Genomic *in situ* hybridization (GISH)**

Kopecky et al. 2006 TAG

Karyotyping

F. pratensis

| 1µm
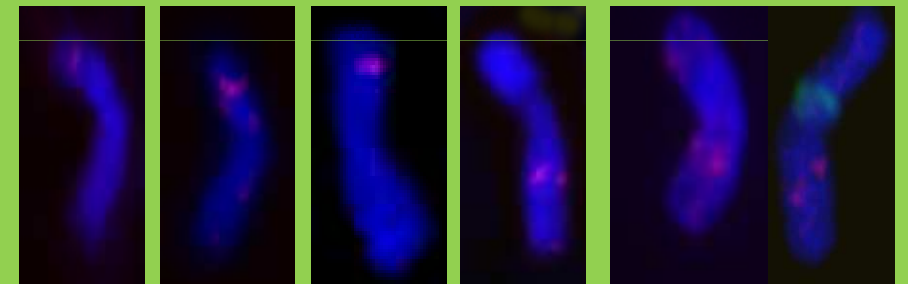
L. multiflorum

| 1µm

■ NOR

Molecular karyotyping using BAC-FISH

BAC clone E18

BAC clone F21

BAC clone E24

BAC clone H22

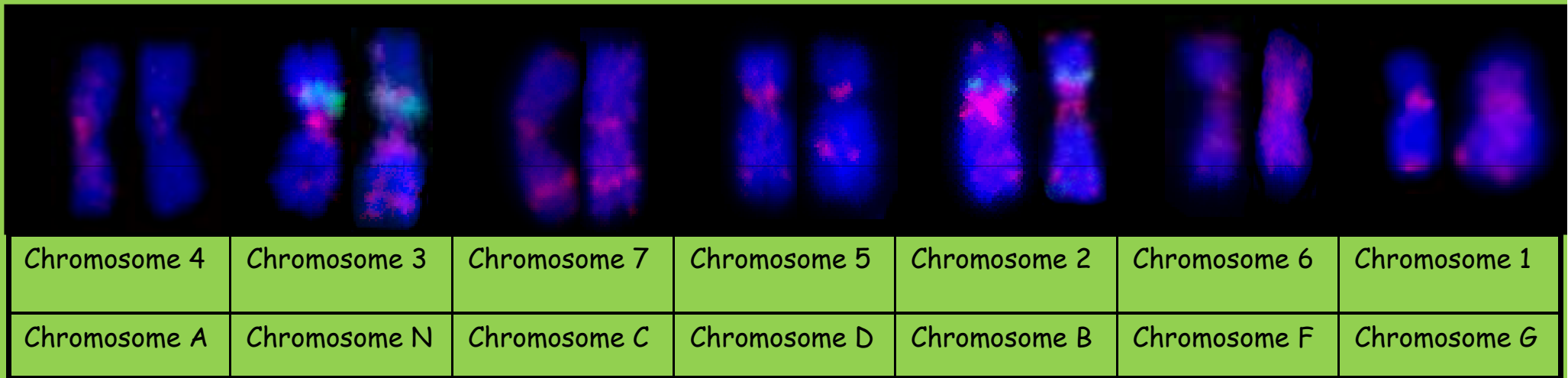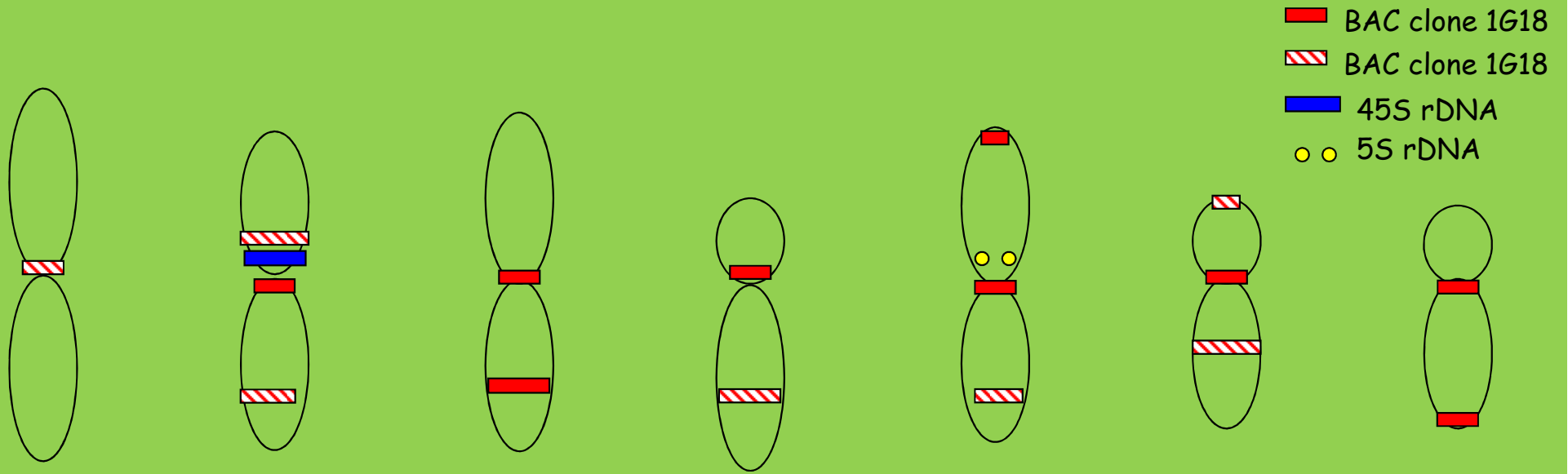BAC clone I2 (red)
45S rDNA (green)



BAC clone J5

BAC clone J22

BAC clone K11

BAC clone N14

BAC clone O16 (red)
45S rDNA green

Kopecky et al. 2008 Chromosome Res

Chromosome Identification in *F. pratensis*

Legend:
- ▬ BAC clone 1G18
- ▨ BAC clone 1G18
- ▬ 45S rDNA
- ○ ○ 5S rDNA

Chromosome 4	Chromosome 3	Chromosome 7	Chromosome 5	Chromosome 2	Chromosome 6	Chromosome 1
Chromosome A	Chromosome N	Chromosome C	Chromosome D	Chromosome B	Chromosome F	Chromosome G

Kopecky et al. 2008 Chromosome Res

Genome size

Species	Cultivar	2C (pg)		1Cx (pg)	1C (Mbp)	Mean CV values	Ploidy level	No. of chromosomes
		Mean	SD					
Festuca pratensis	Kolumbus	6,49	0,078	3,25	3175	2,96	2	14
Festuca pratensis	Patra	13,01	0,050	3,25	5150	2,41	4	28
Festuca arundinacea	Kora	17,45	0,078	2,91	8534	2,80	6	42
Lolium perenne	Handicap	5,36	0,047	2,68	2623	2,16	2	14
Lolium perenne	Korok	11,19	0,057	2,80	5470	2,27	4	28
Lolium multiflorum	Prolog	5,25	0,042	2,62	2567	2,35	2	14
Lolium multiflorum	Lubina	10,99	0,092	2,75	5374	1,96	4	28
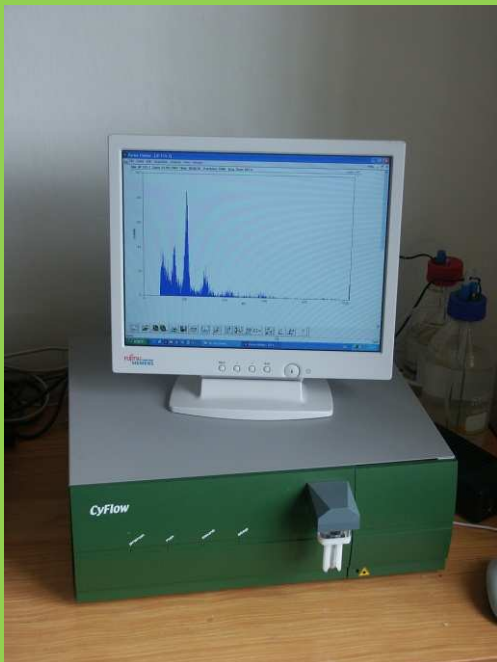
CyFlow flow cytometer (Partec GmbH., Münster, Germany)

1. *Lolium multiflorum* (2x)
2. *Festuca pratensis* (2x)
3. *Pisum sativum* (2x)

Kopecky et al. 2010 Cytogenet Genome Res
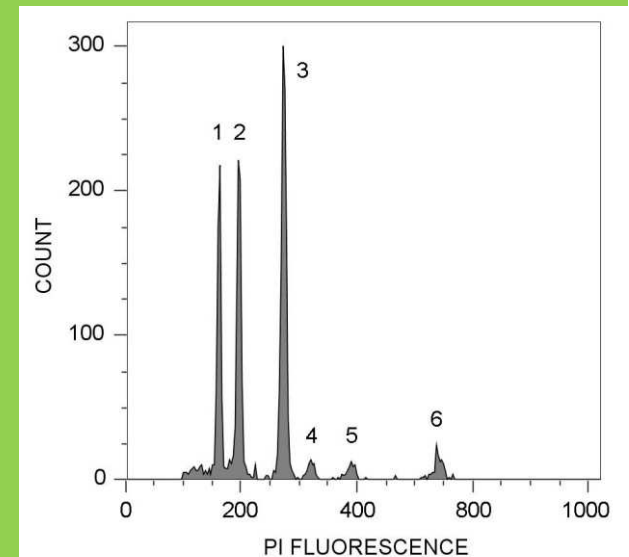
Karyotyping – *Festuca pratensis*

Theoretical flow-karyotype
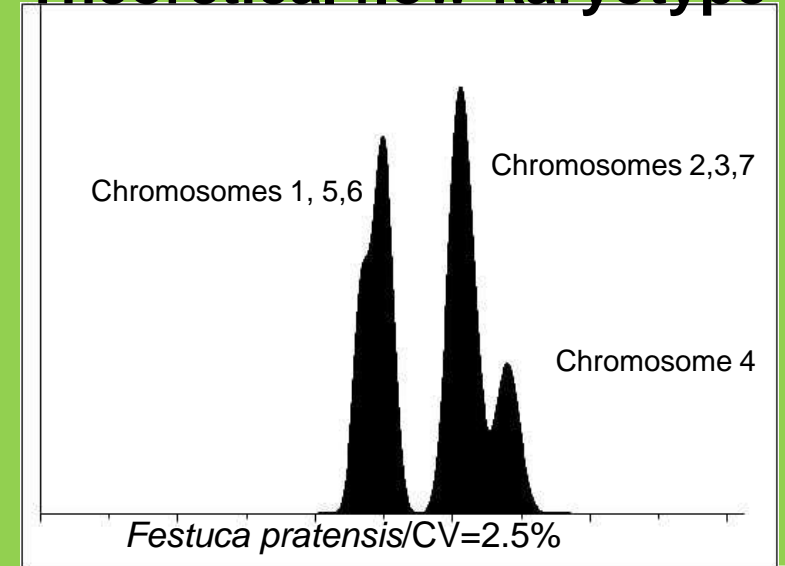
Sorting of individual/groups of chromosomes

Chromosomes 1, 5,6

Chromosomes 2,3,7

Chromosome 4

Festuca pratensis/CV=2.5%

1 2 3 4 5 6 7
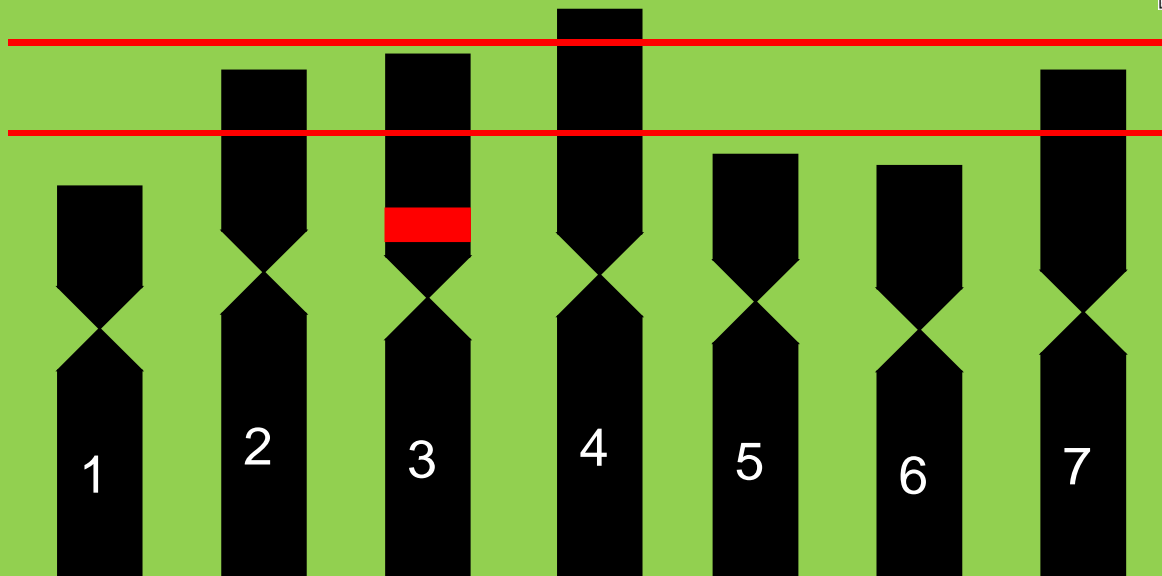
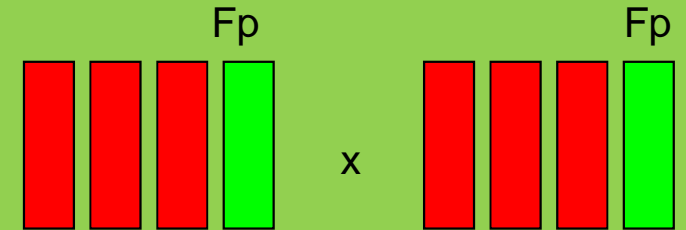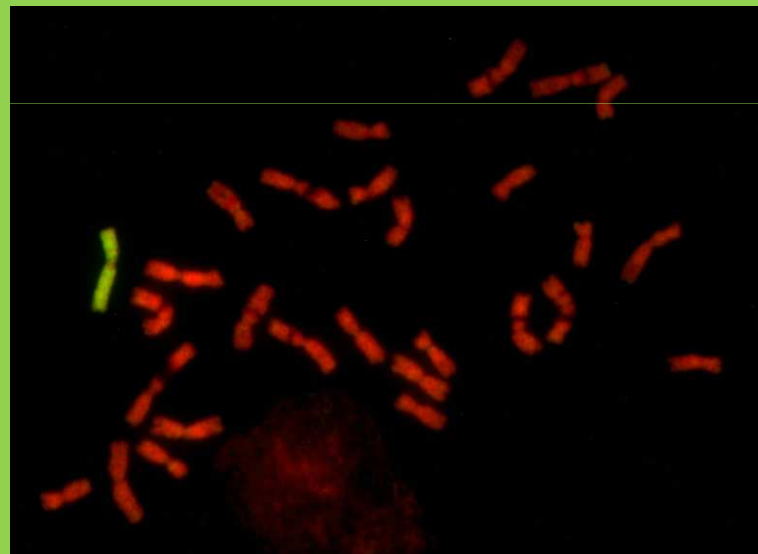# Introgression (substitution) lines

Fp (2n=2x=14) x Lm (2n=4x=28)

F1: (2n=3x=21, FpLmLm) x Lm (2n=4x=28)
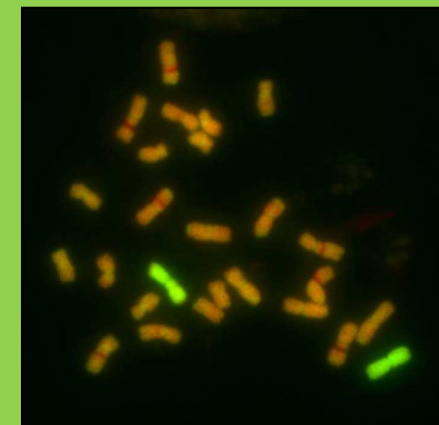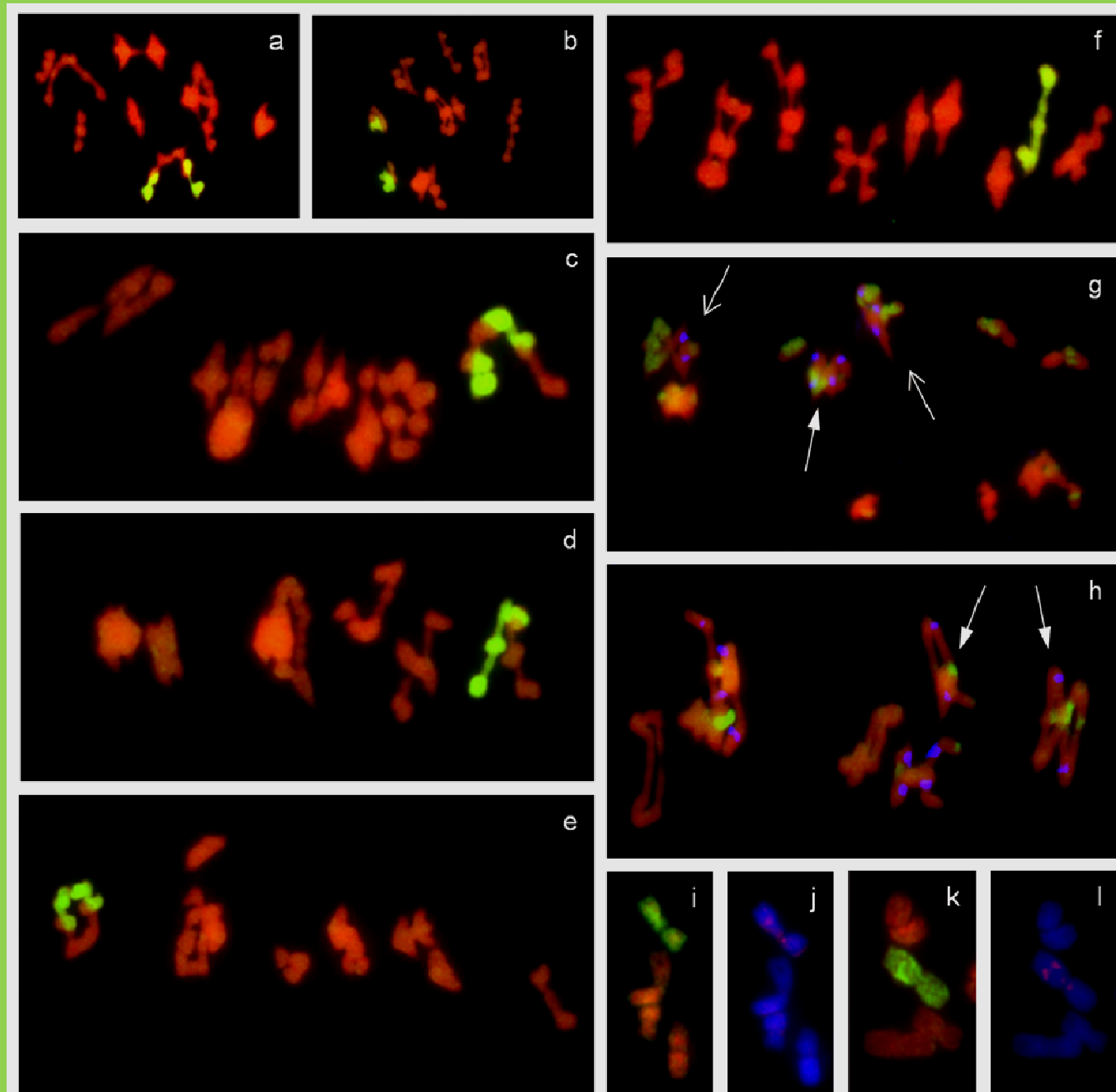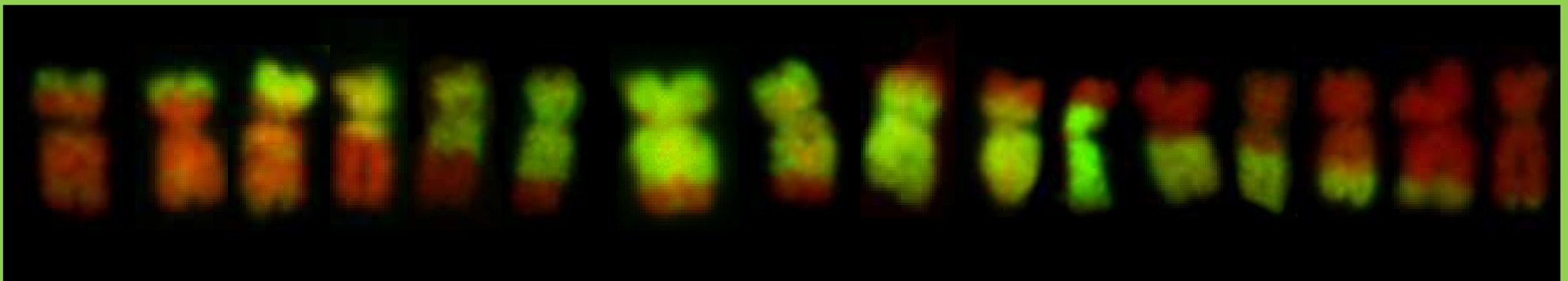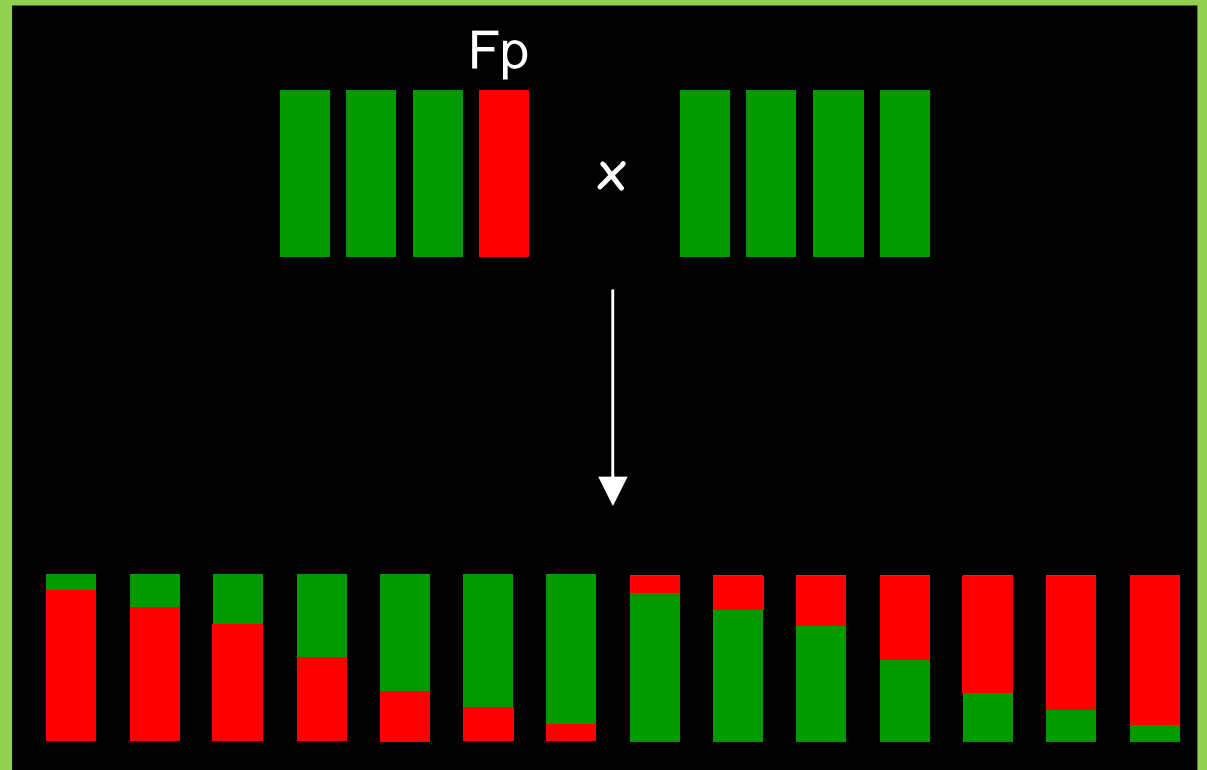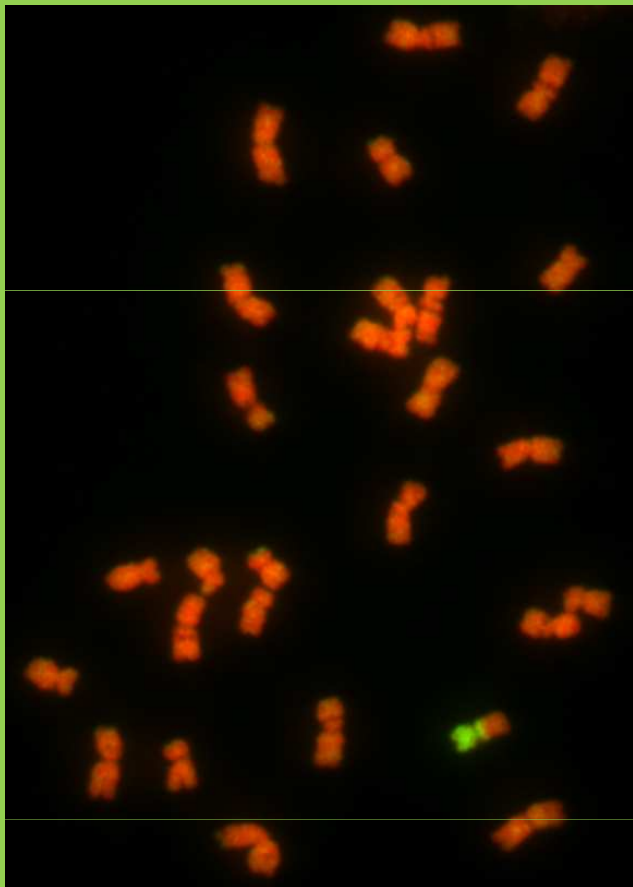
F2: (2n=4x=28, FpLmLmLm) x Lm (2n=4x=28)
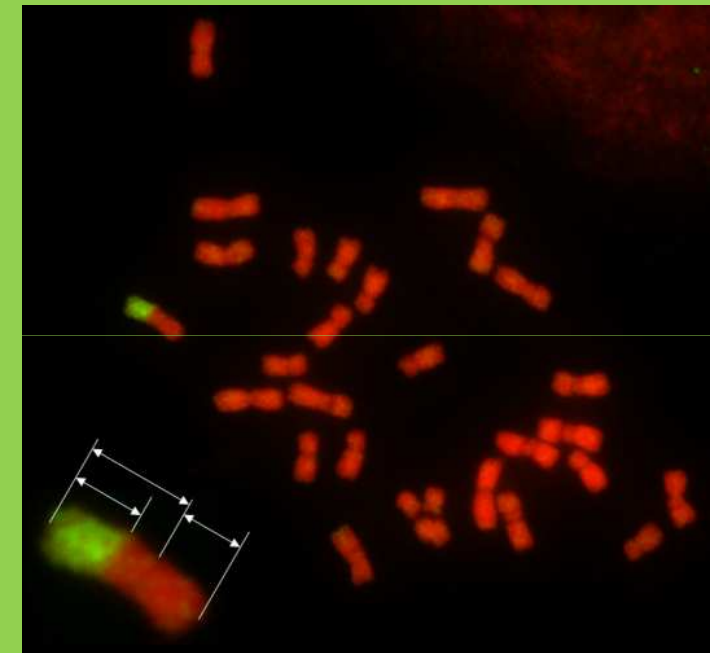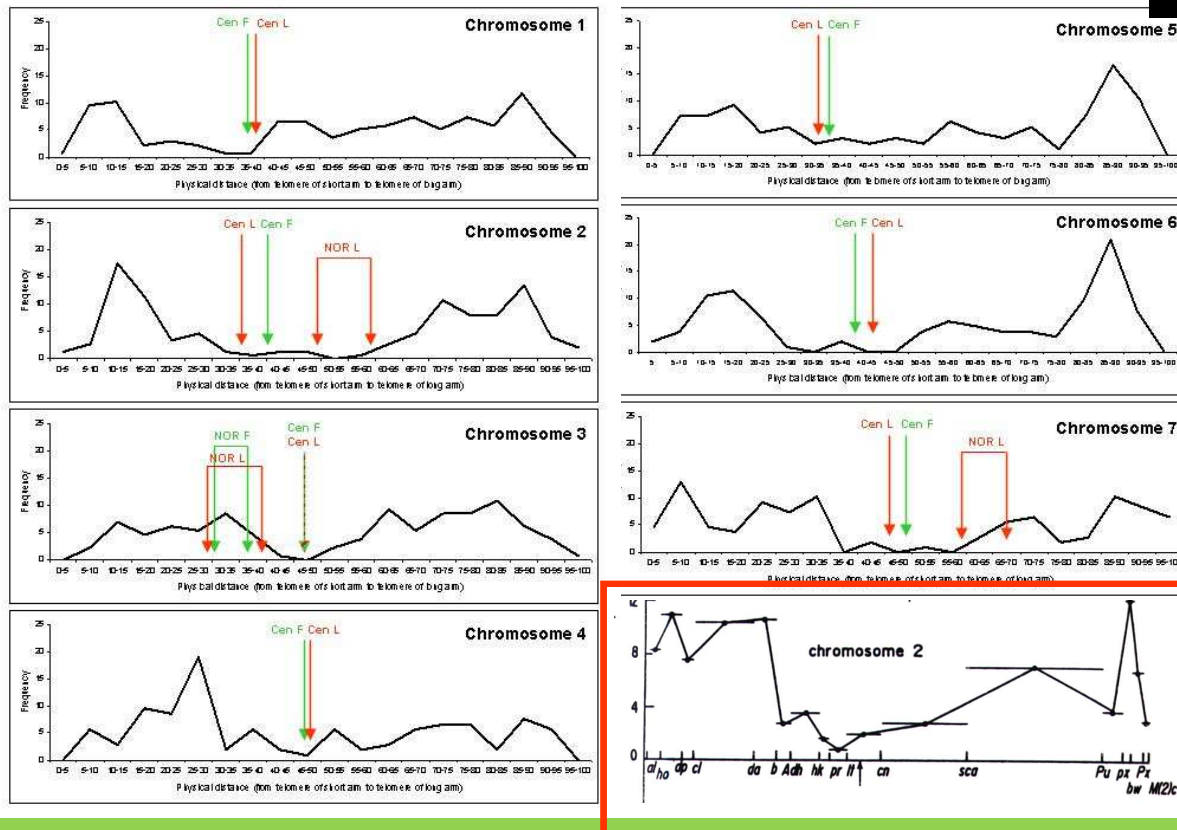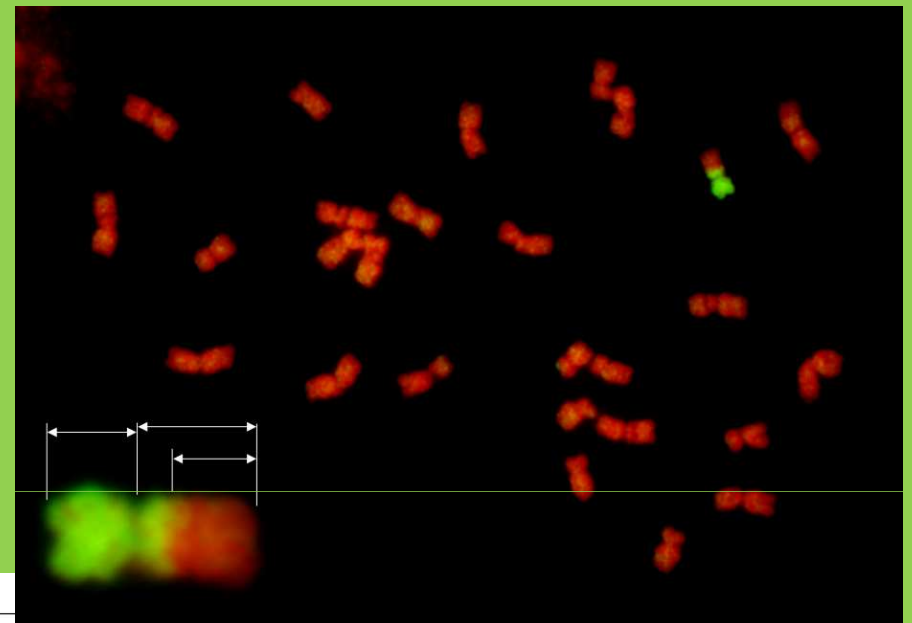
F3 etc.
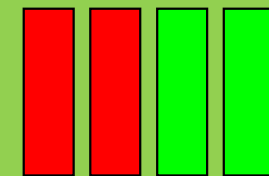
Chromosome pairing in substitution lines
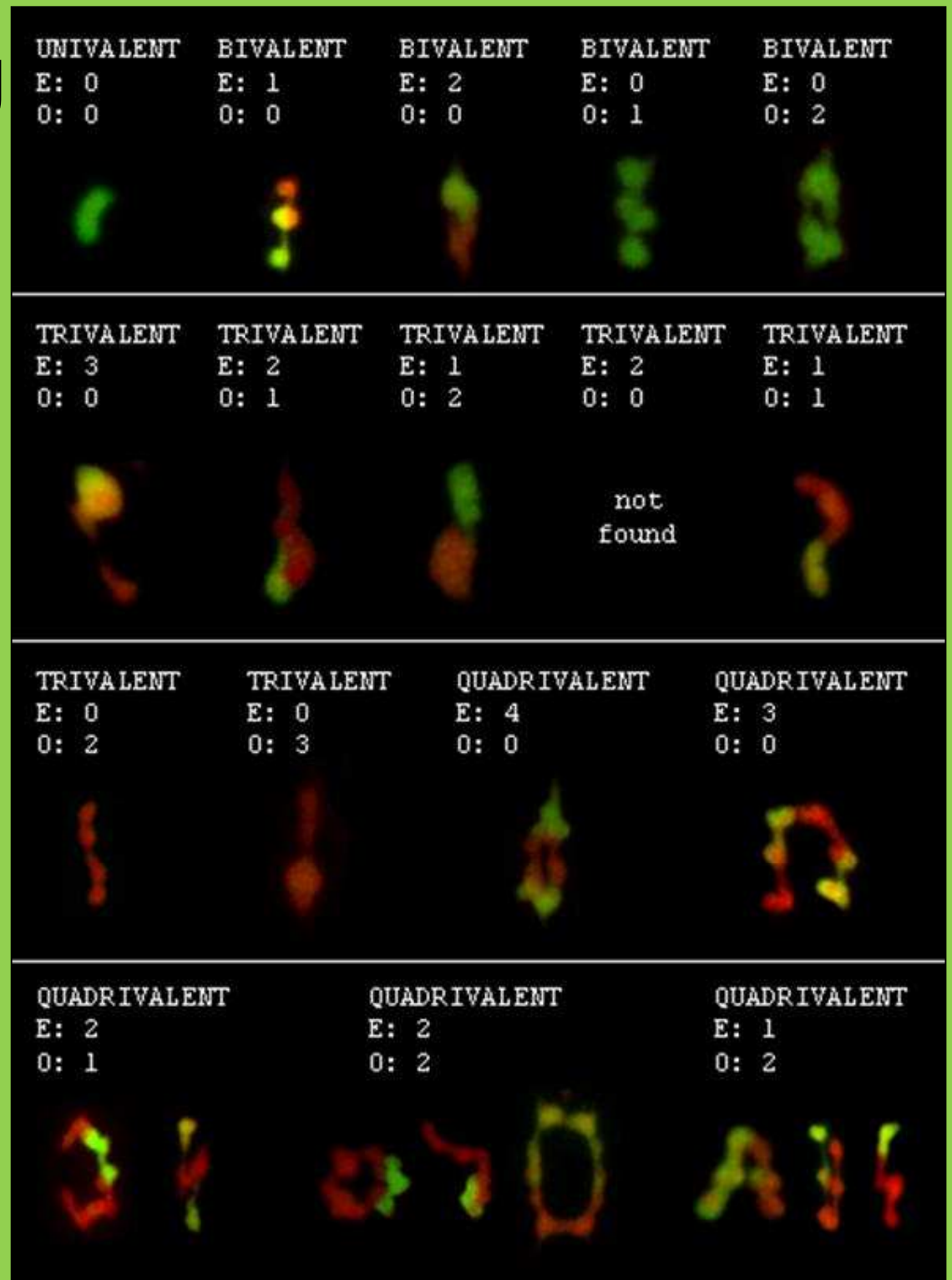
Chromosome pairing in substitution lines

Development of recombinant lines

Fp

Distribution of homoeologous recombination in *Festuca/Lolium*

Kopecky et al. 2010 Cytogenet Genome Res

DArTFest array

A first DArT array for the *Festuca-Lolium* complex

Material: 5 species
- 40 accessions of *Lolium perenne* (2x, 4x)
- 40 accessions of *L. multiflorum* (2x, 4x)
- 40 accessions of *Festuca pratensis* (2x, 4x)
- 40 accessions of *F. arundinacea* (6x)
- 7 accessions of *F. glaucescens* (4x)

Array: 7680 probes

Number of polymorphic markers: 3884

Cost: ~ $50,000

Genetic diversity

Based on the analysis of 2637 DArT markers

Genetic diversity

(*F. pratensis*)

Parents of mapping population
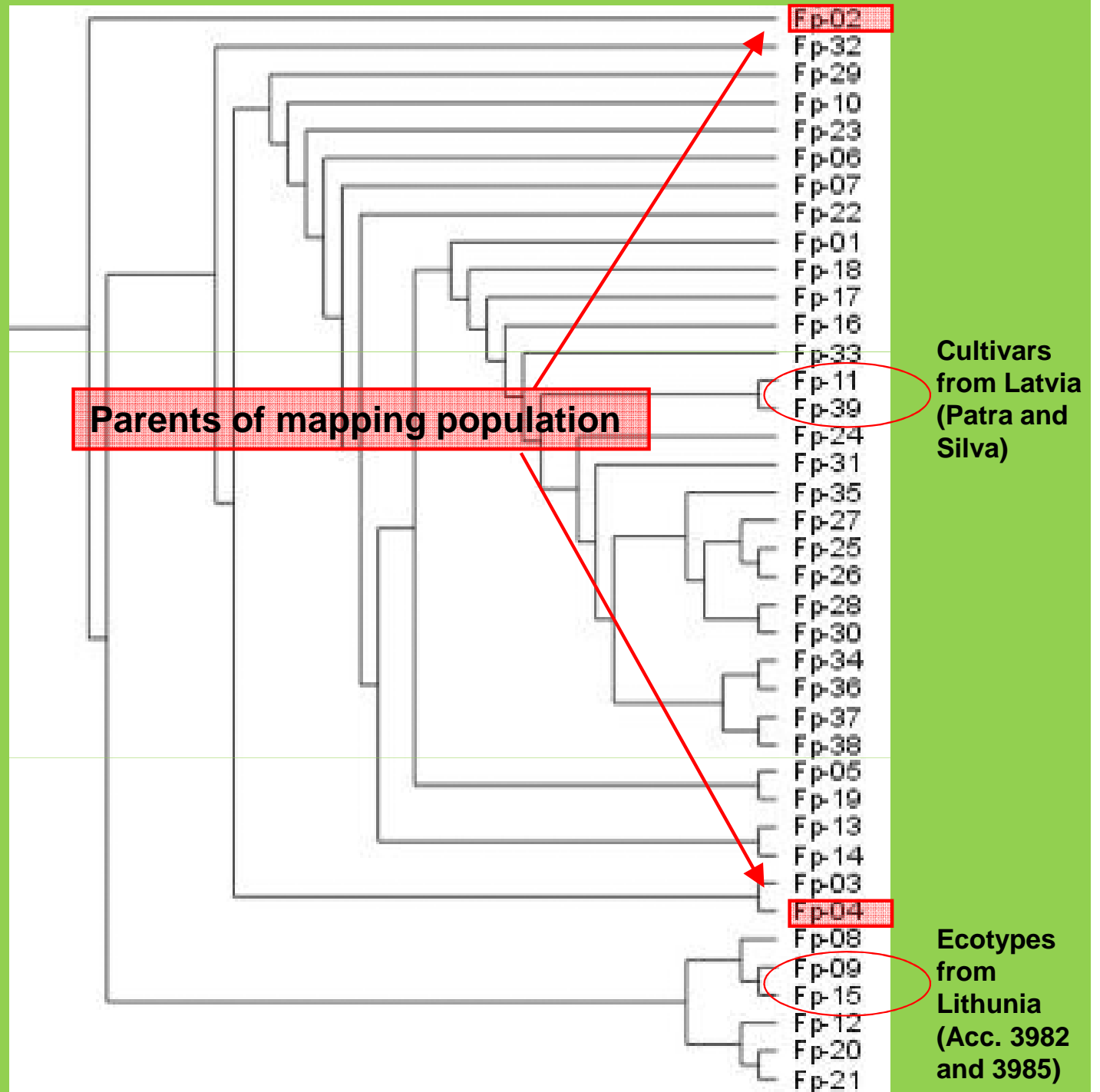
Cultivars from Latvia (Patra and Silva)

Ecotypes from Lithunia (Acc. 3982 and 3985)
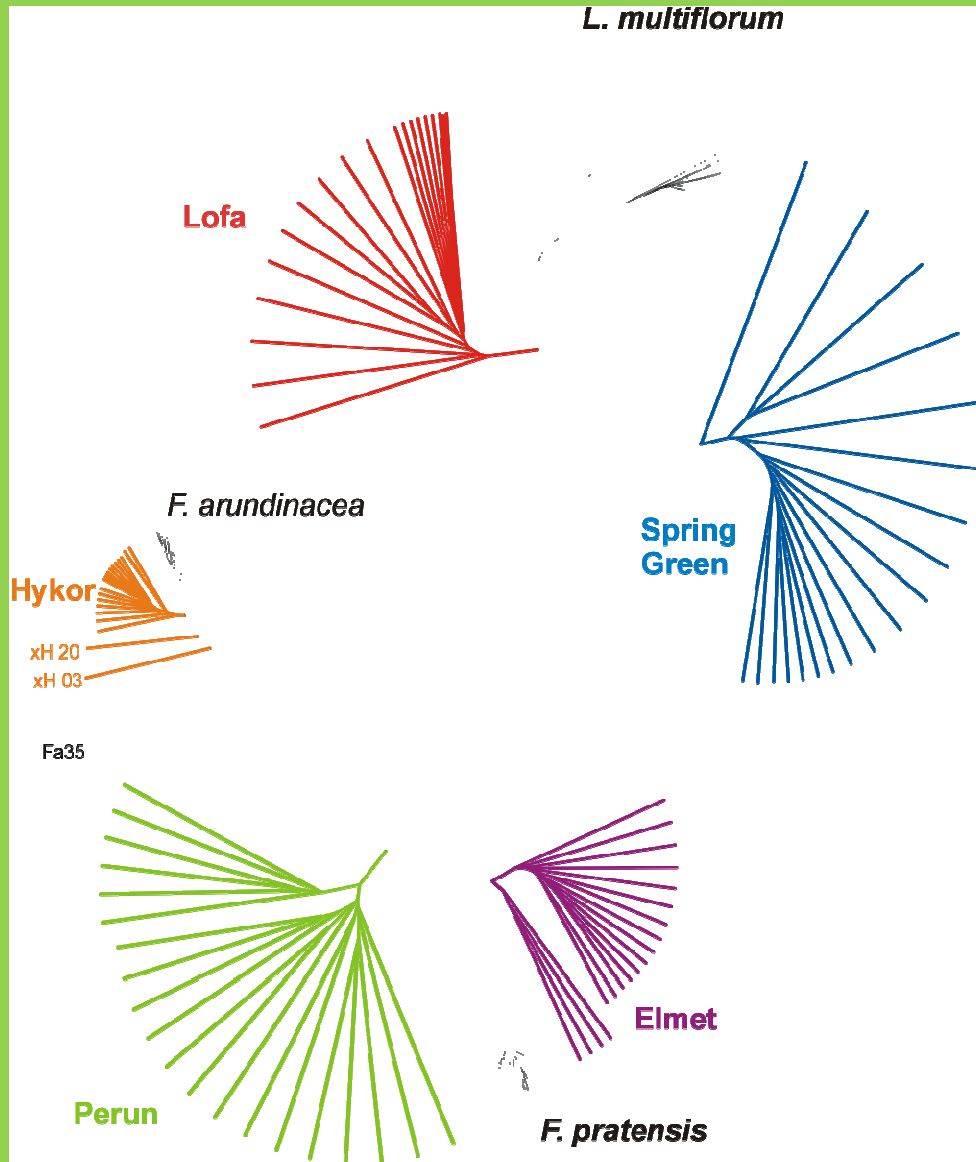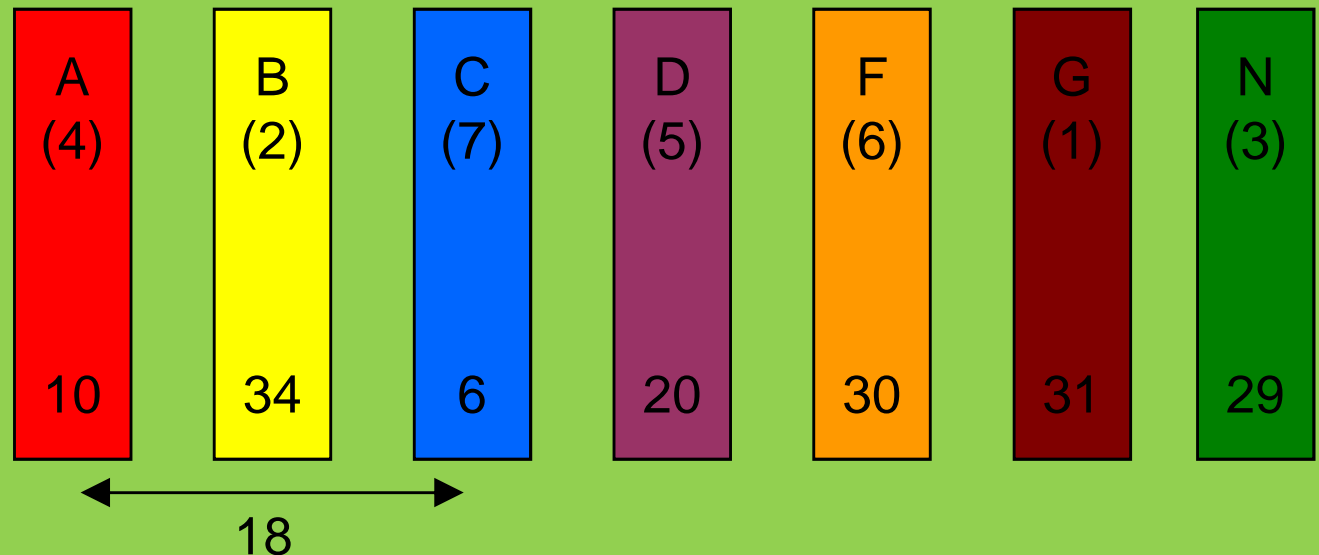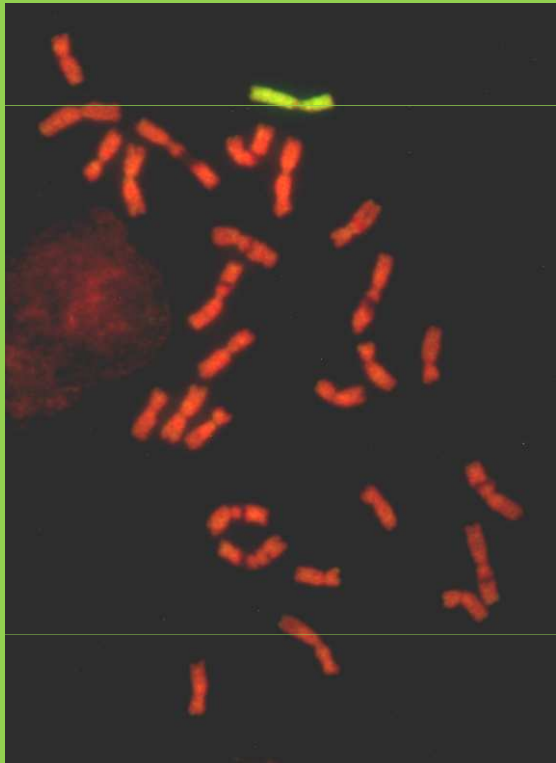
Diversity in hybrids

Individual plants

Bulked samples
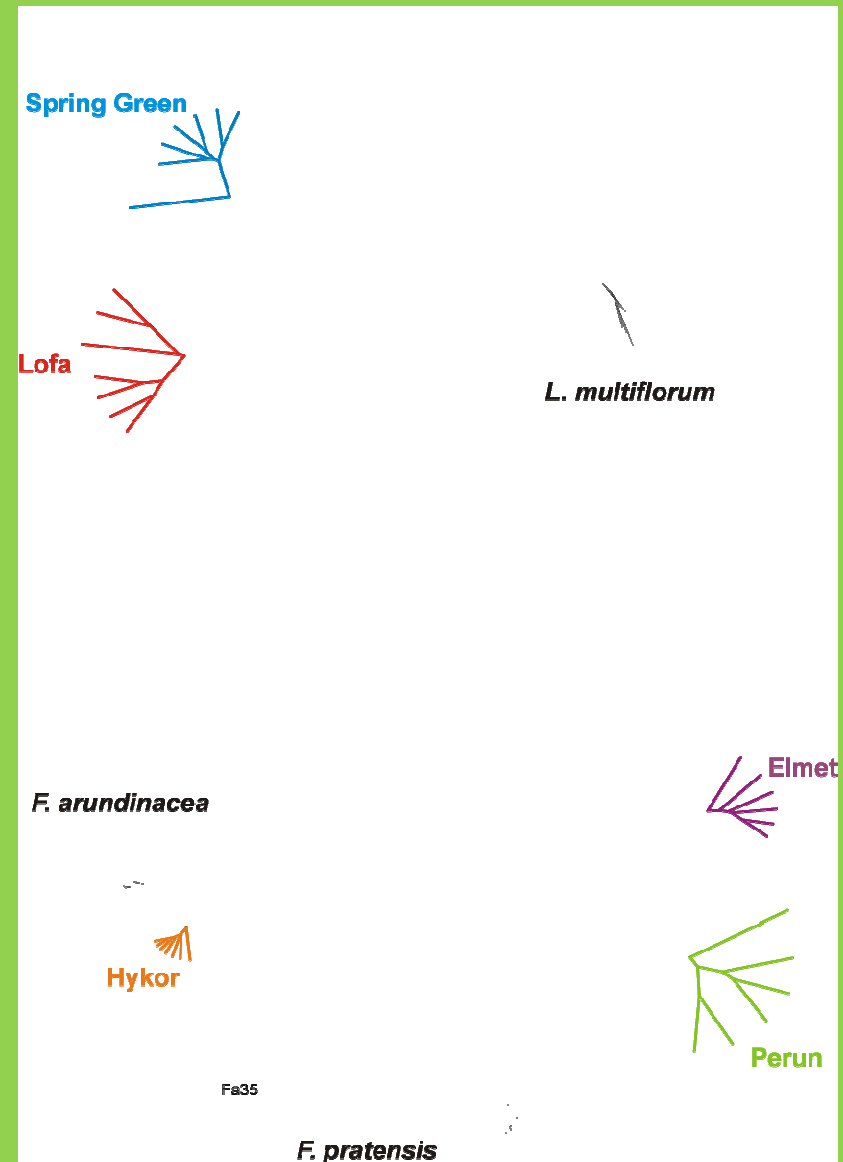
Based on 1471 informative DArT markers

Anchoring DArT markers to individual Fp chromosomes

A (4) 10
B (2) 34
C (7) 6
D (5) 20
F (6) 30
G (1) 31
N (3) 29

18

=160 markers (out of 288 *F. pratensis* positive and *Lolium* negative)

Genetic mapping

	Festuca pratensis		Lolium multiflorum	
	pre-DArT markers	DArT	pre-DArT markers	DArT
LG1	61	23	63	67
LG2	26	10	43	73
LG3	34	22	49	73
LG4	68	22	61	106
LG5	70	12	43	71
LG6	49	27	35	63
LG7	65	32	68	76
Total	**373**	**148**	**352**	**529**

Sequencing

So far, sequenced over 1000 DArT markers

Results for 621 markers placed on genetic maps:

303,297 bp (=488.4 bp)
399 markers were singletons (64.3%)
222 markers were redundant (assigned to 90 marker bins)
= 489 non-redundant markers/bins

44 markers contained repetitive elements (=7.1%)

368 (59.3%) DArT markers with significant homology to expressed sequences

163 (26.2%) DArT markers with significant homology to known and hypothetical proteins

379 DArT markers (293 non-redundant bins) were identified as potentially gene-derived sequences
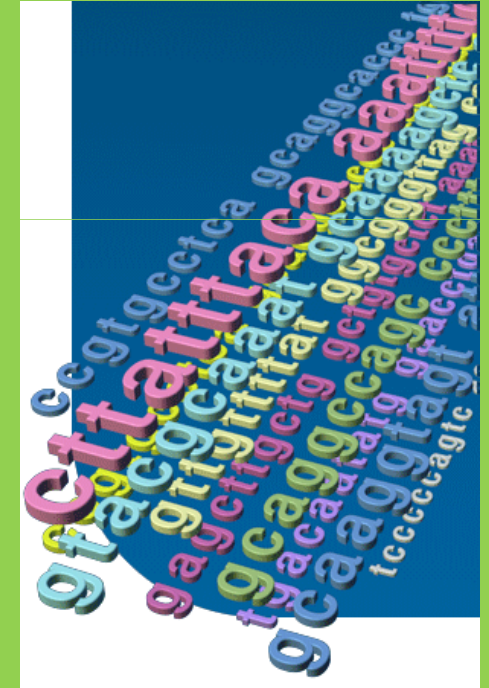
Marker Assisted Selection

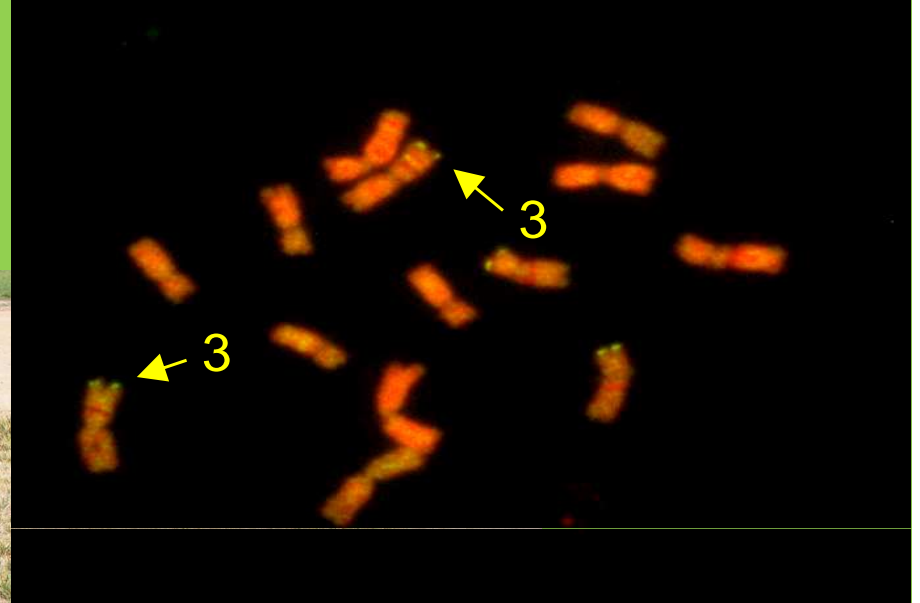|	Tolerant	Susceptible
Freezing	51	45
Snow mold	47	49
Rust	71	14

Extreme dry-down
UCR, Riverside CA
early October
(no rain, no irrigation since late July;
temperatures often exceeding 100ºF)

F. arundinacea

Festulolium

3

3

Following June:
no irrigation for 11 months; 3.5" total rainfall
Last survivors

Conclusion: extreme drought tolerance
associated with introgression on 3S

Future work in Festulolium

Converting DArT markers associated with traits of interest to PCR

Check the specificity of DArT markers with more tolerant/susceptible plants

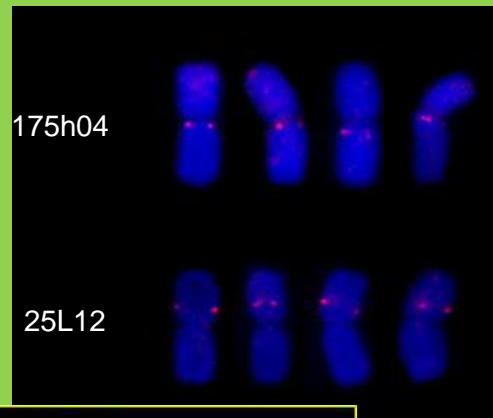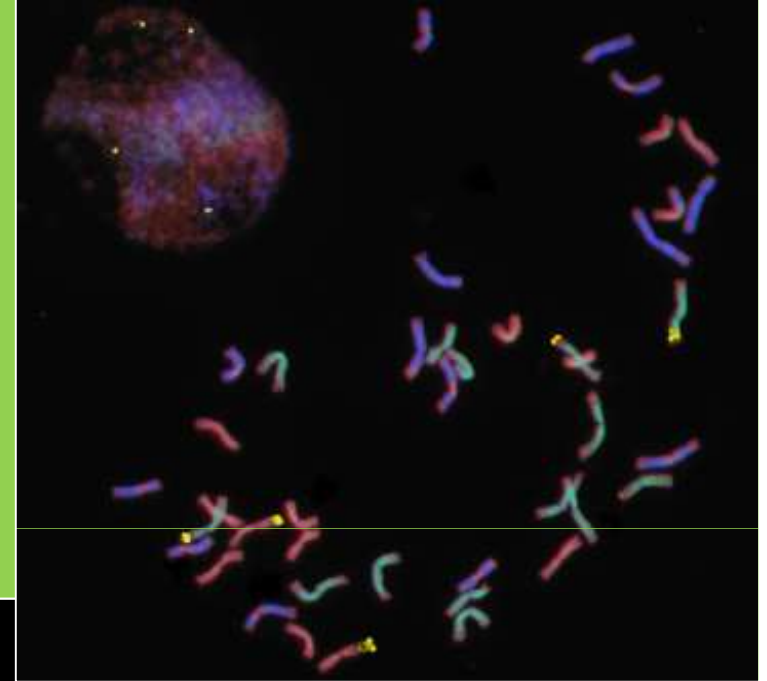Phenotyping of all substitution lines

Developing of all seven turf-type diploid substitution lines *L. perenne* x *F. pratensis*
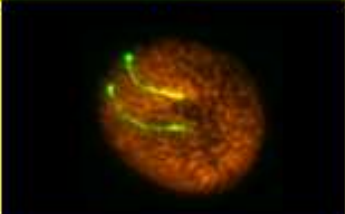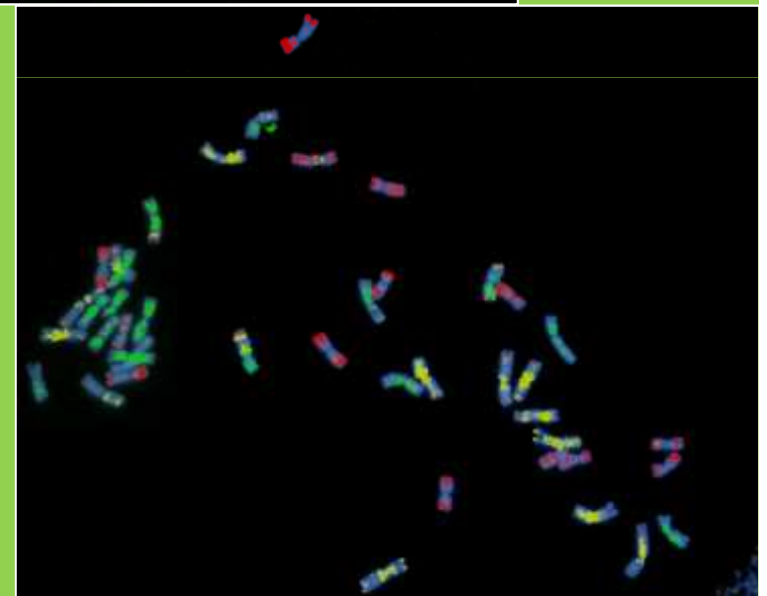
Phenotyping them

Selection and intercross with aim to release **new superior turf-type hybrid cultivar**

Side projects

- Chromosome pairing in wheat and rye and their hybrids (Adam J. Lukaszewski UCR)

- custom made chromosome constructs for sorting in wheat/rye (with AJLukaszewski, UCR)

- Genomic contig localization in wheat via BAC-FISH (Ming-Cheng Luo and Jan Dvorak UCD)

- Fine mapping of cDNA clones in barley (Nils Stein IPK)

-Structure and evolution of weeds *Elytrigia* (*Elymus*) and *Thinopyrum* (Vaclav Mahelka, BI, Prague)

- Selection of drought/heat tolerant turf Festulolium (with J. Baird and AJLukaszewski)

175h04

25L12

Translocation	Interphase nuclei	
	Ph1	*ph1b*
1RS.1BL		

Acknowledgements

Acknowledgements

Potential in Buffalograss and Blue Grama

Buffalograss (*Buchloe dactyloides*)

2n=2x=20, 2n=4x=40, 2n=6x=60
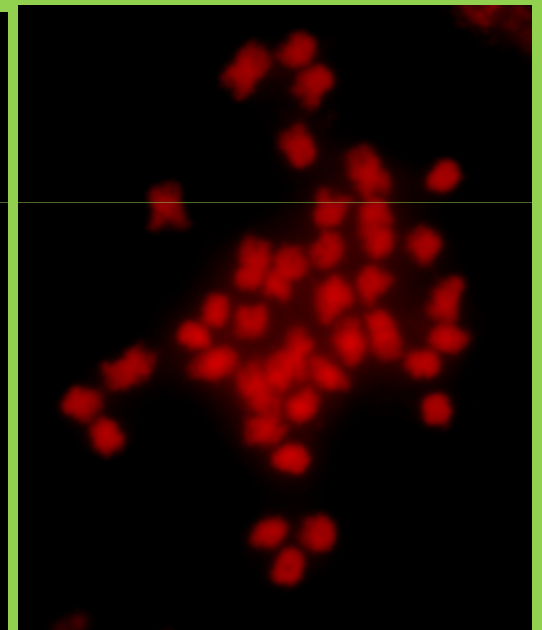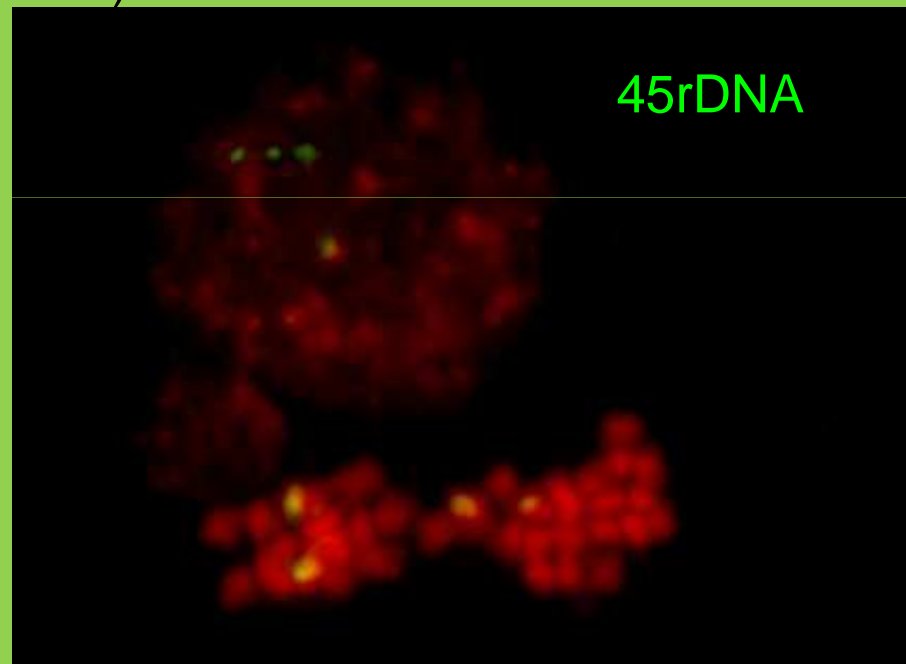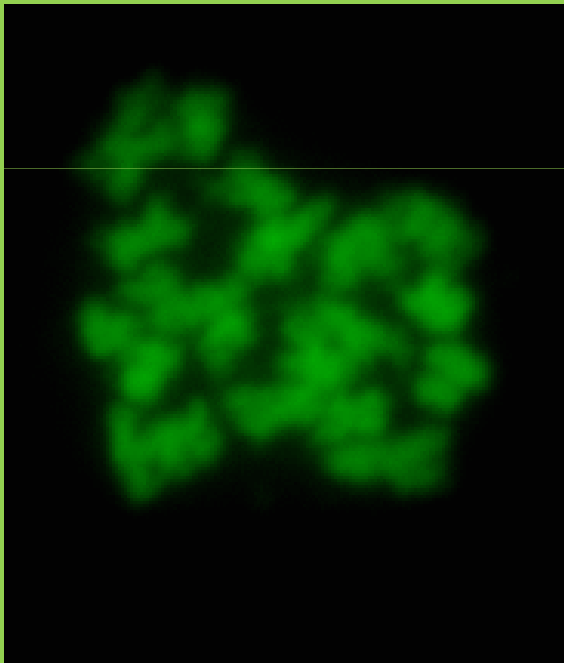
Limited set of molecular markers
(ISSR, SSR, RAPD, SRAP)

Blue Grama (*Bouteloua gracilis*)

2n=2x=20, **2n=4x=40**, 2n=6x=60

45rDNA

You guys could have developed grasses with larger chromosomes!

Development of DNA array

Based on my experience, DArT array would be suitable for genomic studies in buffalograss and blue grama.

About 100,000 sequenced tags would be used.

Diversity studies: select potential parents of mapping population(s)

Mapping population(s): cover the highest possible genetic diversity or target specific trait(s).

Genetic map(s) constructed.

Phenotyping (chinch bug tolerance, …..).

Markers associated with traits of interest identified and converted to PCR (cheaper and more effective). Markers used for MAS to accelerate breeding. DArT array may offer cultivar protection (PVP) (in essence, fingerprinting).

Molecular karyotype(s)

BAC library constructed. BAC clones used as a source of molecular cytogenetic markers to identify individual chromosomes.

Molecular karyotype constructed and chromosomes sorting tested.

Physical map(s)

BAC library **fingerprinted** and **physical map(s)** developed.

If sorting successful, individual chromosomes (or entire genome) can be **sequenced**.

My main goals:

- To develop, register and propagate new superior cultivars

-To incorporate molecular methods into cultivar development

- Investigate biological phenomena of general significance

- To motivate new people (students) to work on grasses

- To collaborate with other Faculty and offer them my
    knowledge and skills