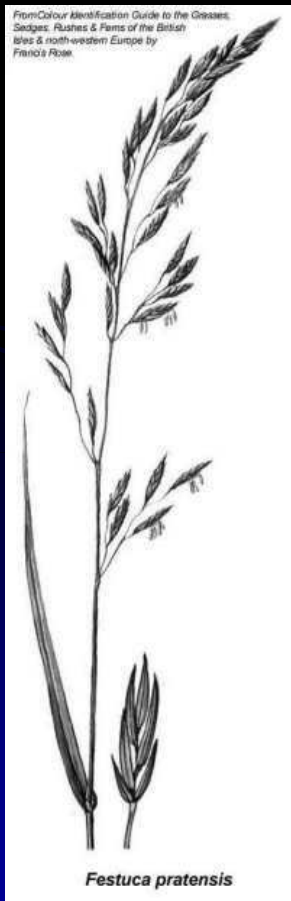


Genome mapping within the *Festuca-Lolium* complex



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FESTUCA - LOLIUM COMPLEX

- Ones of the most important forage and turf species
- Cross of the species of both genera results in fertile hybrids
- Hybrids display agronomically important attributes of both genera
- Chromosomes of both genera frequently pair and recombined with each other
- Chromosomes of both genera can be distinguished by GISH



	Lm	Lp	Fp	Fa
Rapid establishment from seed	+	+/-	-	-
Good production in seeding year	+	+/-	-	-
Palatability	+	+/-	+	-
Persistency	-	+	+	+
High tillering density	-	+	-	-
Resistance to treading	-	+	-	-
Winter hardiness	-	+/-	++	+
Drought tolerance	-	-	+	++
Continued high production after second harvest year	-	-	+	+



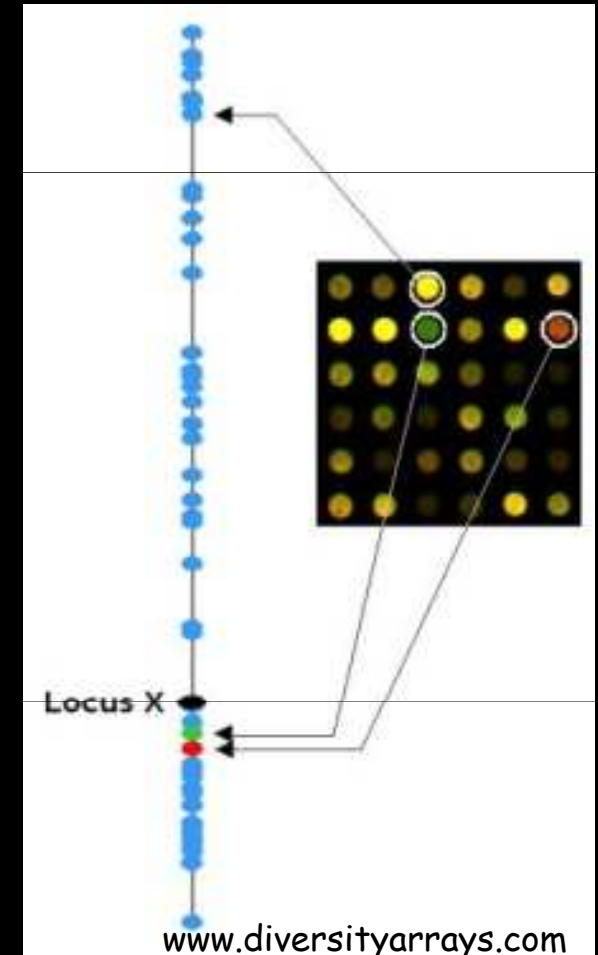
DArT technology

Diversity Array Technology

- Developed by Dr. Andrzej Kilian (Canberra, Australia) (Jaccoud et al., 2001)
- microarray technology
- dominant markers
- positives: high number of markers, anonymous markers (can be sequenced)
- negatives: low polymorphism in some species

Main use:

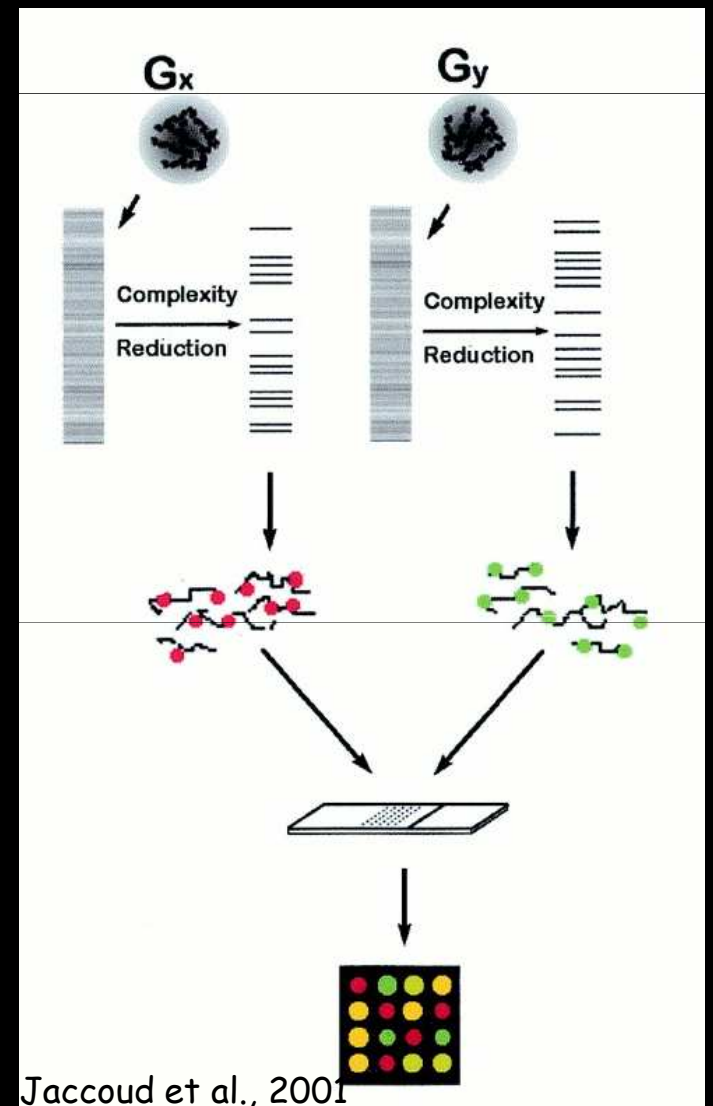
- identification of QTL
- simultaneous marker-assisted selection for several traits
- evaluation of genetic diversity
- varietal identification of crops
- monitoring the composition of complex DNA samples (microbial, populations, etc.) - also in hybrids
- methylation profiling



DArT technology

DArT technology consists of several steps:

- Complexity reduction of DNA
- Library creation
- Microarray from libraries
- Hybridization of fluoro-labelled DNA
- Scanning of slides for hybridization signal (confocal laser)
- Data extraction and analysis (DArT software)



DArTFest

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

Development of array:

Material: 5 species

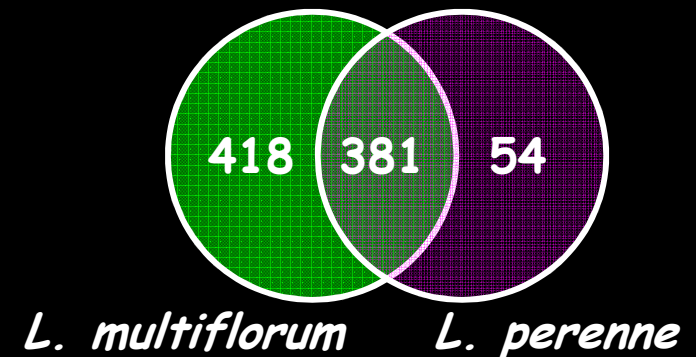
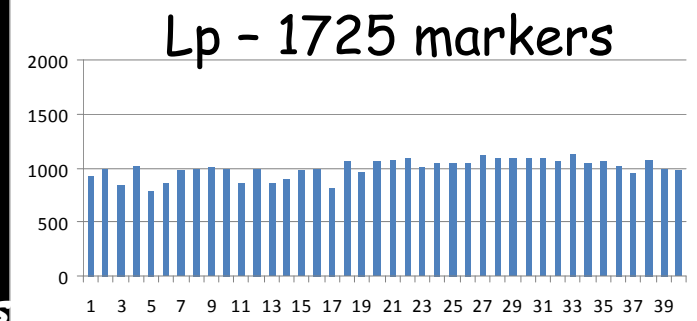
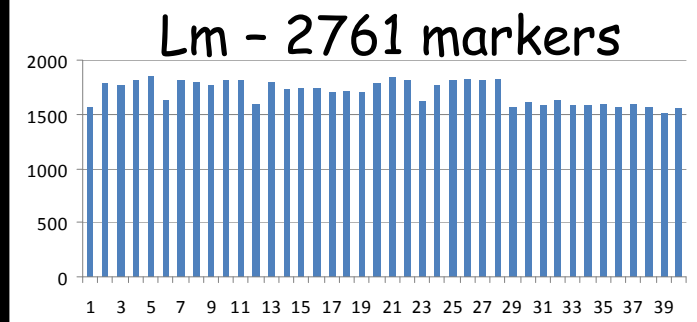
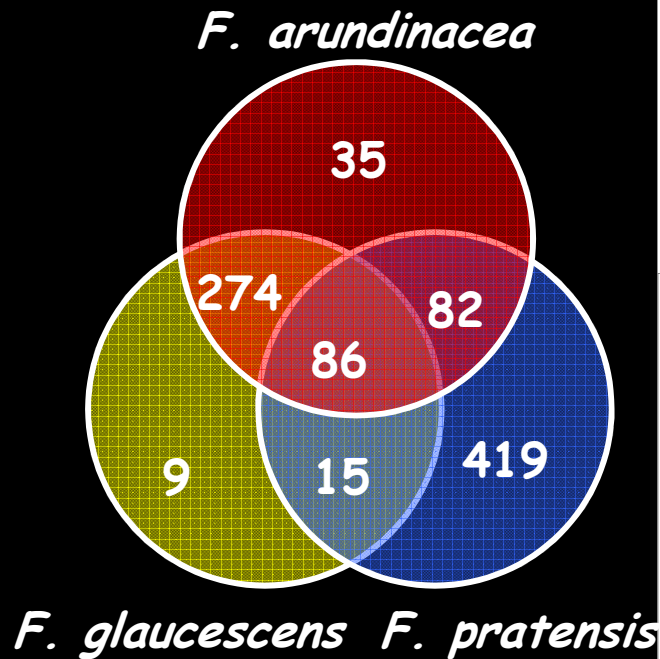
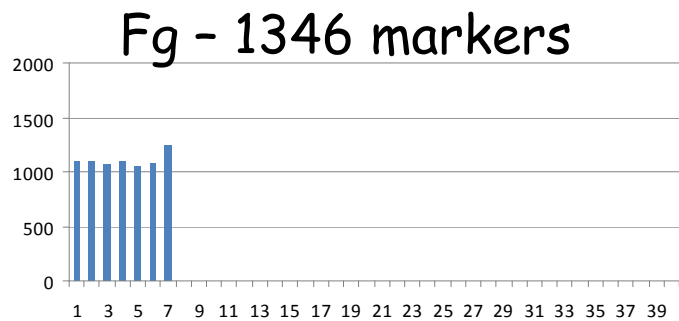
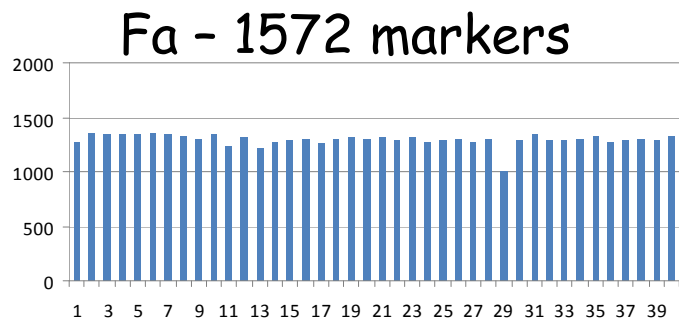
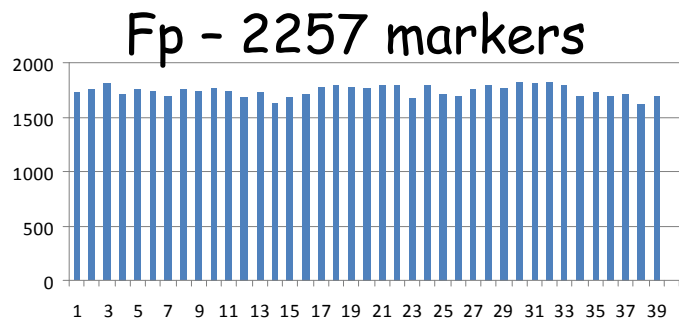
- 40 accessions of *Lolium perenne*
- 40 accessions of *L. multiflorum*
- 40 accessions of *Festuca pratensis*
- 40 accessions of *F. arundinacea*
- 7 accessions of *F. glaucescens*

Array: 7680 probes

Number of polymorphic markers: 3884

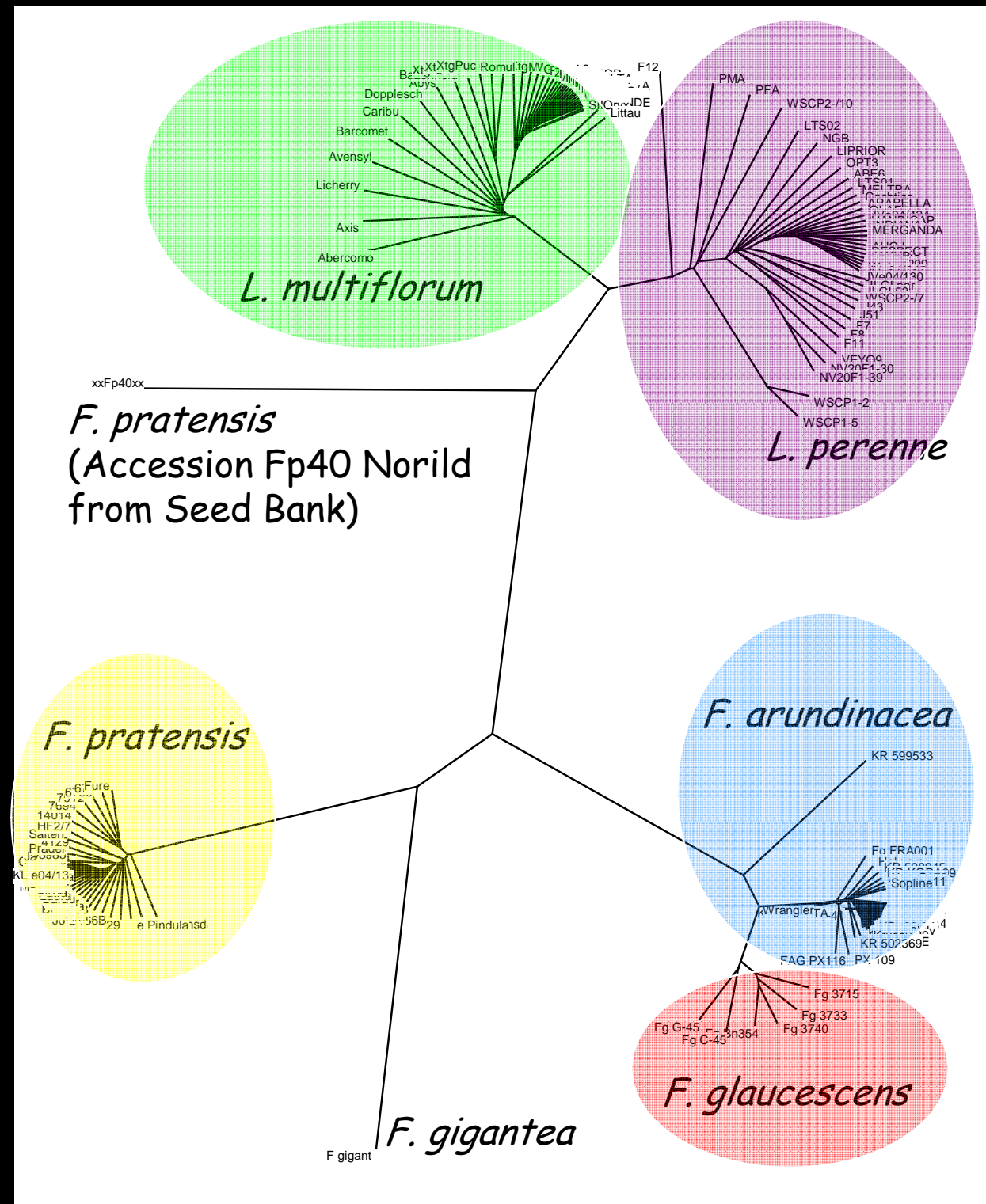


DArTFest: Genus- and species-specific markers

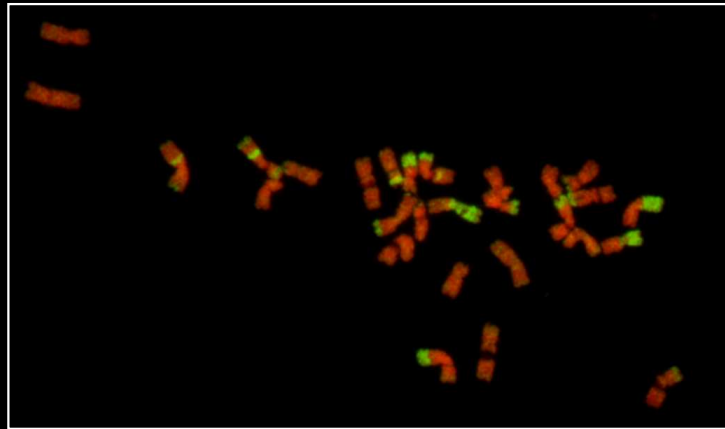


DArTFest: Genetic diversity

Based on the analysis of 2637
DArT markers



DArTFest in hybrids



L. multiflorum × *F. pratensis*

1203

1558

699

L. multiflorum × *F. glaucescens*

937

869

477

L. multiflorum × *F. arundinacea*

828

983

589

L. perenne × *F. pratensis*

826

899

448

L. perenne × *F. arundinacea*

778

947

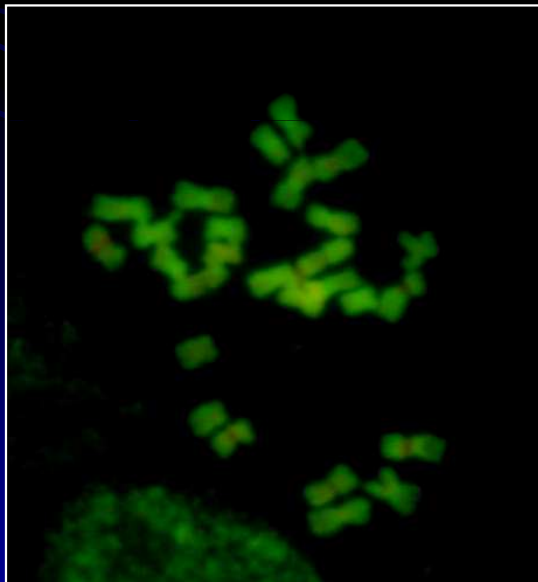
625

L. multiflorum × *L. perenne*

289

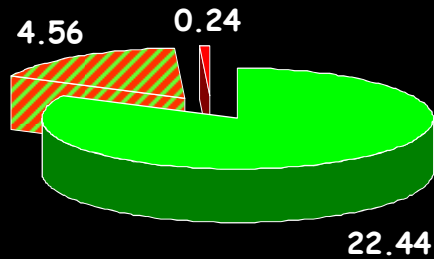
1522

203

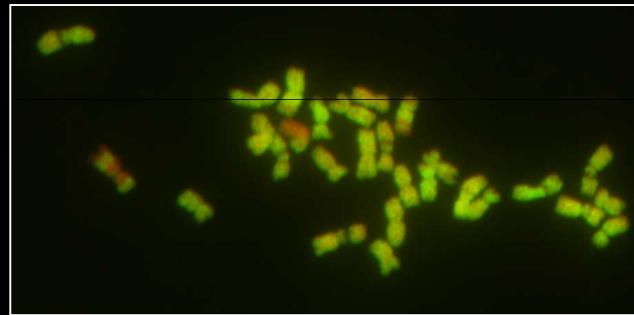


DArTFest in hybrids

Spring Green



GISH



DArT

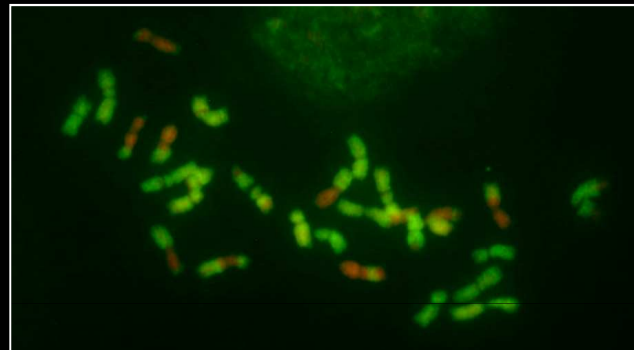
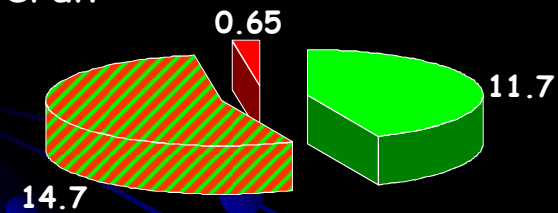
Lm

Fp

468

65

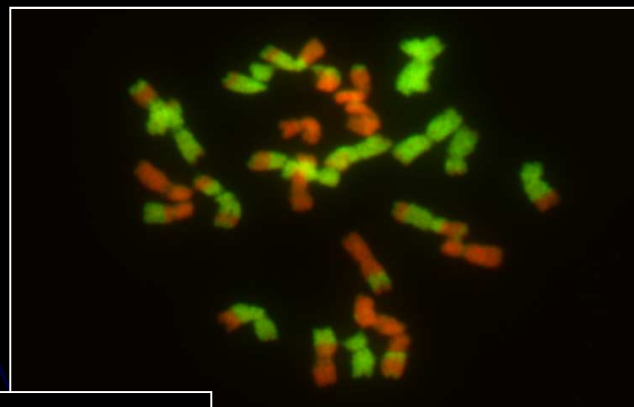
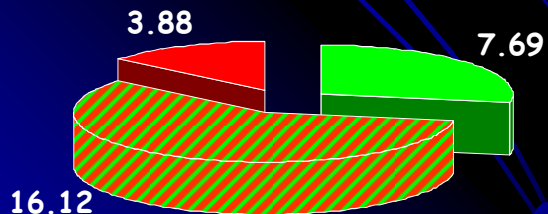
Perun



470

342

Elmet



359

410

■ *L. multiflorum* ■ Recombination LmxFp ■ *F. pratensis*

DArTFest: physical mapping

Development of materials for the mapping

$$F_p (2n=2x=14) \times L_m (2n=4x=28)$$



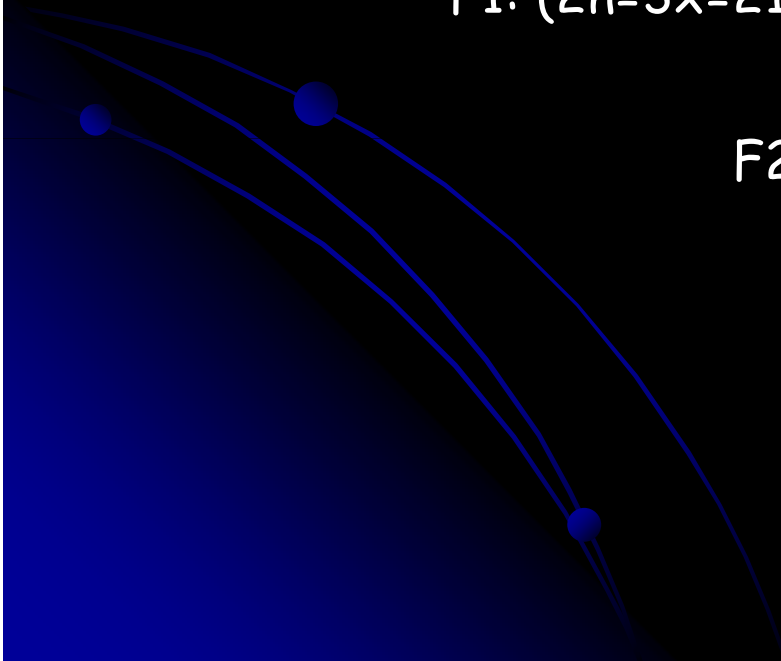
$$F_1: (2n=3x=21, F_p L_m L_m) \times L_m (2n=4x=28)$$



$$F_2: (2n=4x=28, F_p L_m L_m L_m) \times L_m (2n=4x=28)$$

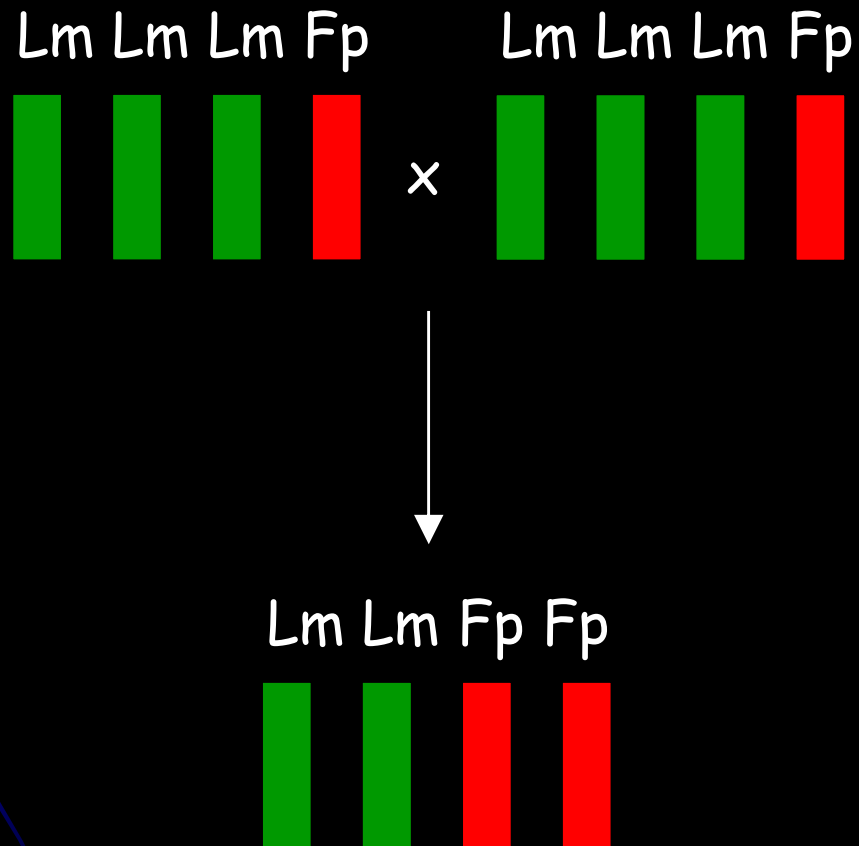


$$F_3 \text{ etc. } (2n=4x=28; 27L_m+1F_p)$$

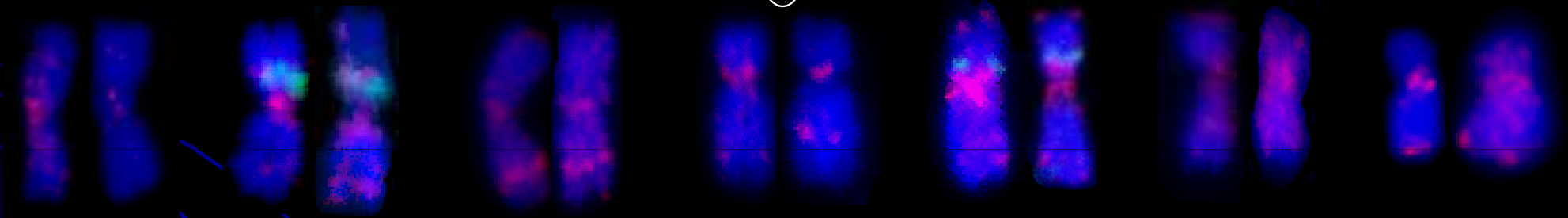
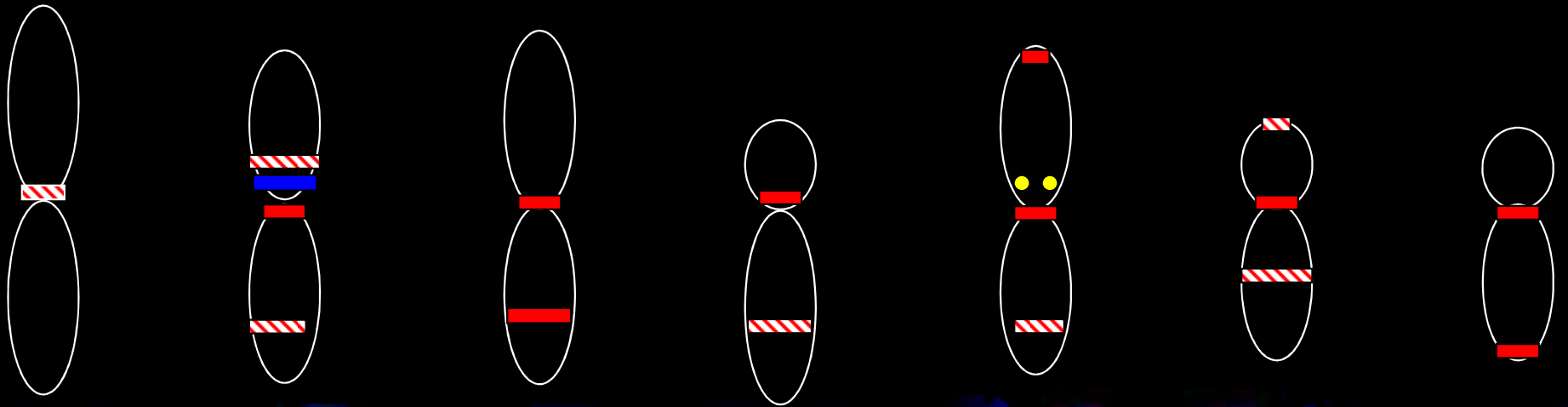


DArTFest: physical mapping

Development of chromosome substitution lines



Cytogenetic mapping in *Festuca pratensis*



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

— BAC clone 1G18

▨ BAC clone 1G18 (weak signal)

— 45S rDNA

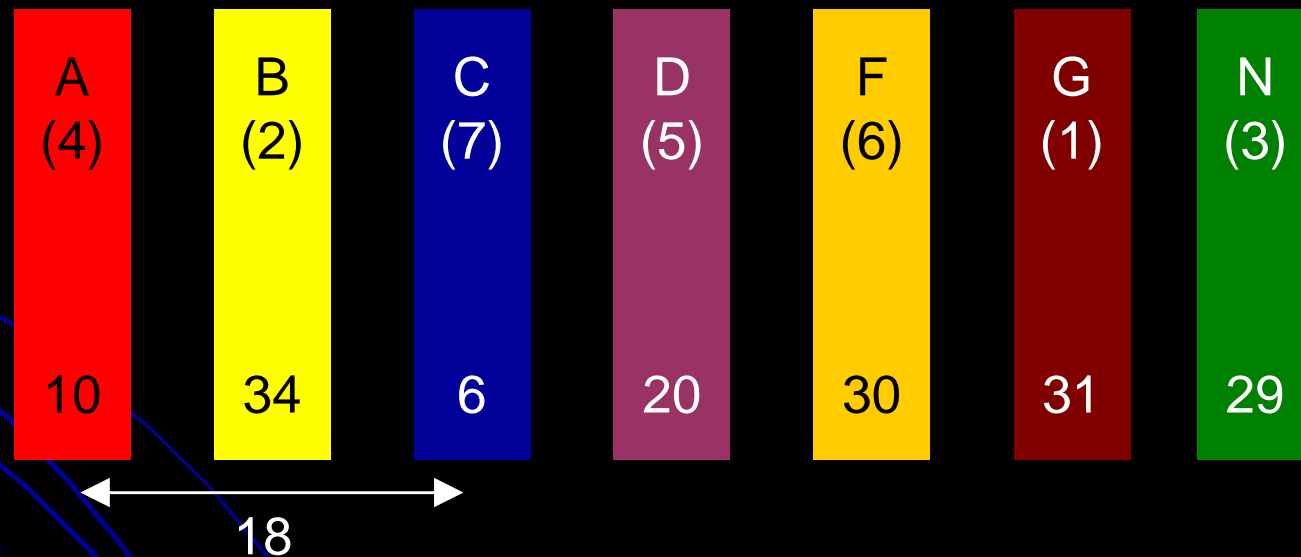
●● 5S rDNA

DArT*Fest*: physical mapping

Development of chromosome substitution lines

Chromosome	Monosomic lines $2n=4x=28; 27Lm+1Fp$	Disomic lines $2n=4x=28; 26Lm+2Fp$
A (4)	4	5
B (2)	6	1
C (7)	12	7
D (5)	4	0
F (6)	2	4
G (1)	11	4
N (3)	7	1

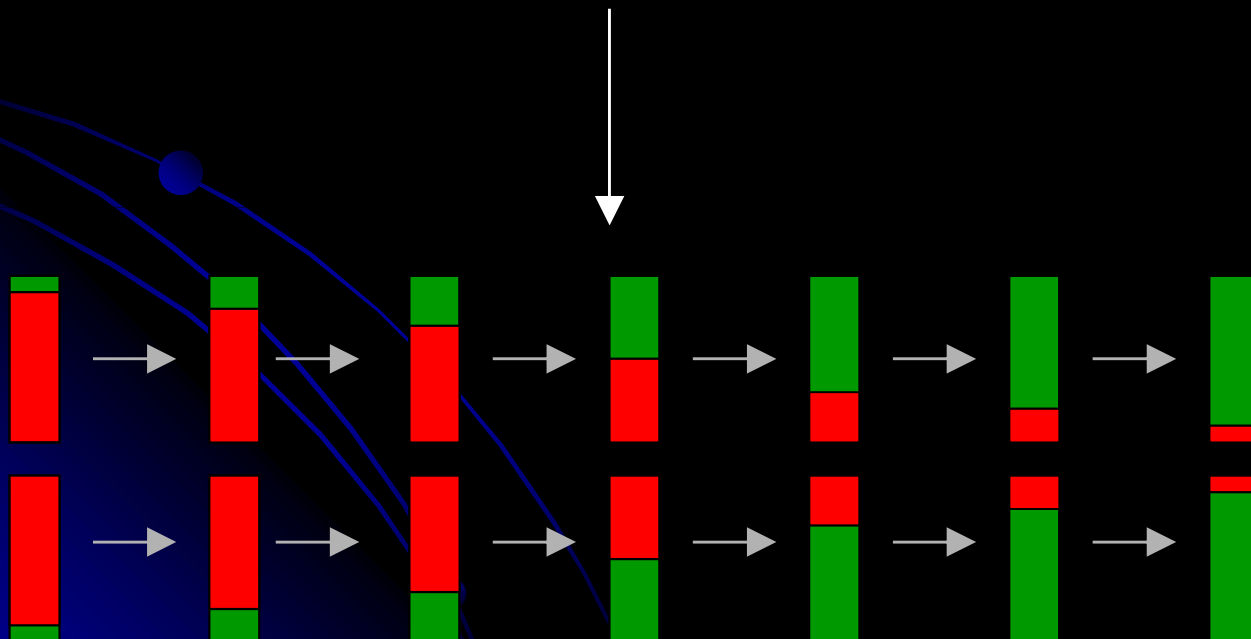
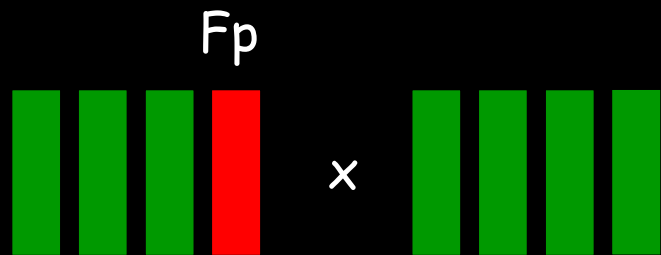
DArTFest: Anchoring DArT markers to individual Fp chromosomes



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

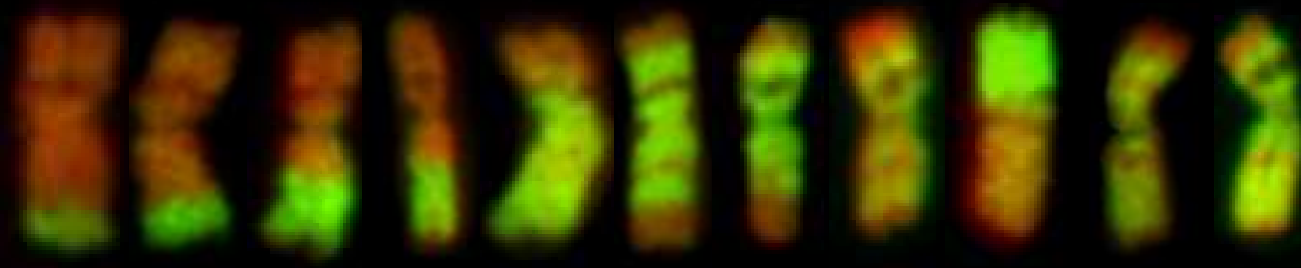
DArT*Fest*: physical mapping

Development of recombination lines

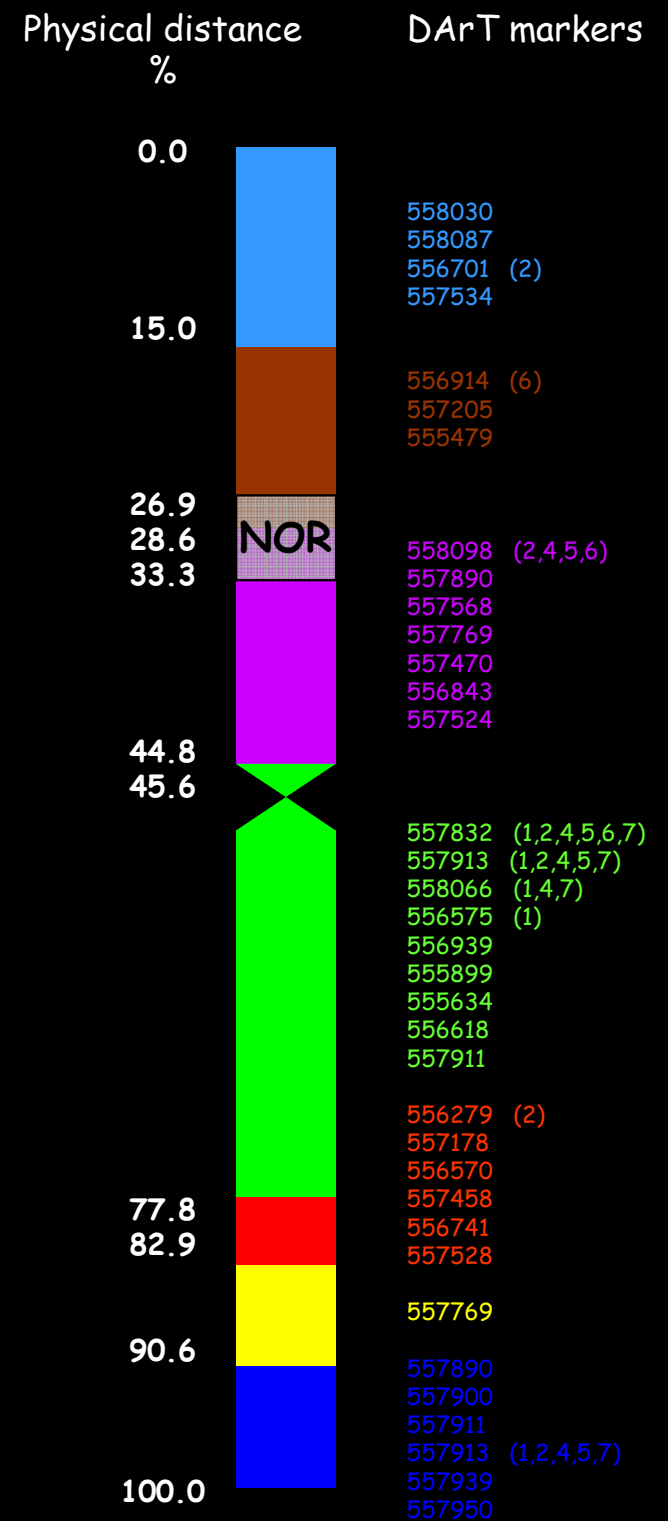
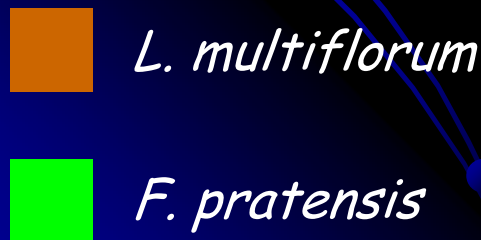


- More than 30 lines have been developed for each F_p chromosome
- 14 recombination lines for chromosome N (3) were used in a pilot study

DArTFest: Anchoring DArT markers to chromosome bins



Chromosome N (3) of *F. pratensis*



*) detected in other chromosomes

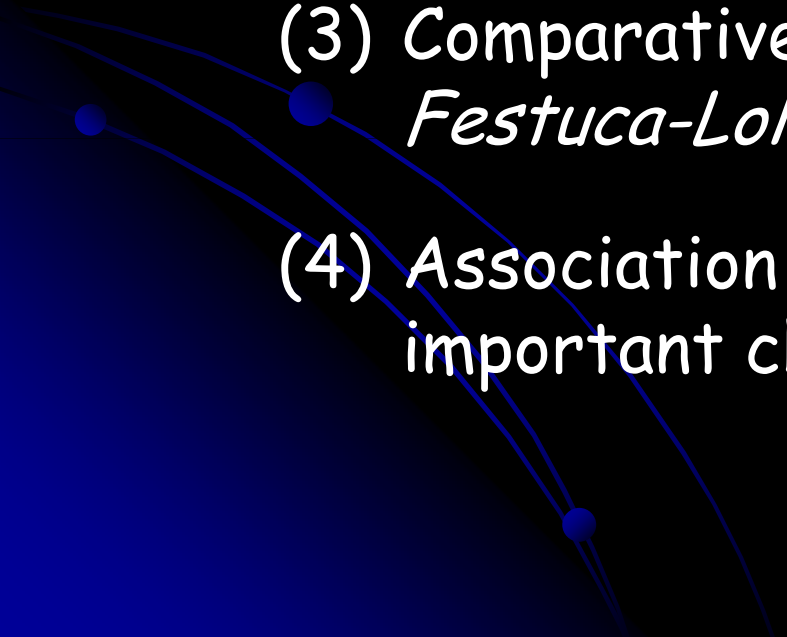
Conclusions

We developed the first DArT array (DArTFest) for *Festuca-Lolium* complex

The array contains 3884 markers and can be used for:

- Analysis of genetic diversity
- Genotyping (including DArT fingerprinting of cultivars)
- Analysis of genomic constitution of hybrids
- Genetic and physical mapping

Future work

- (1) Screening of mapping populations and establishment of genetic maps with DArT markers
 - (2) Integration of genetic and bin maps
 - (3) Comparative analysis of species within the *Festuca-Lolium* complex
 - (4) Association of markers with agronomically important characters
- 

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Thank you for your attention

