Chromosome Research in the *Festuca-Lolium* Complex

David Kopecký

*Laboratory of Molecular Cytogenetics and Cytometry*
*Institute of Experimental Botany of the AS CR*
*Olomouc, Czech Republic*
*http://olomouc.ueb.cas.cz*
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)

<table>
<thead>
<tr>
<th><strong>Some agronomic characteristics</strong></th>
<th>Lm</th>
<th>Lp</th>
<th>Fp</th>
<th>Fa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid establishment from seed</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seed production</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Palatability</td>
<td>++</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Persistence</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resistance to treading</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Winter hardiness</td>
<td>-</td>
<td>+/-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Drought tolerance</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Sustained productivity</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
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**

**L. multiflorum**

Molecular karyotyping using BAC-FISH

- BAC clone E18
- BAC clone E24
- BAC clone F21
- BAC clone H22
- BAC clone J5
- BAC clone K11
- BAC clone J22
- BAC clone N14
- BAC clone O16 (red)
- 45S rDNA (green)

Kopecky et al. 2008 Chromosome Res
Chromosome Identification in *F. pratensis*

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>BAC clone 1G18</th>
<th>45S rDNA</th>
<th>5S rDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 1</td>
<td>BAC clone 1G18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 2</td>
<td>45S rDNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 3</td>
<td>5S rDNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 4</td>
<td>BAC clone 1G18</td>
<td>45S rDNA</td>
<td>5S rDNA</td>
</tr>
<tr>
<td>Chromosome 5</td>
<td>45S rDNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 6</td>
<td>5S rDNA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kopecky et al. 2008 Chromosome Res
## Genome size

<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivar</th>
<th>2C (pg)</th>
<th>1Cx (pg)</th>
<th>1C (Mbp)</th>
<th>Mean CV values</th>
<th>Ploidy level</th>
<th>No. of chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Festuca pratensis</em></td>
<td>Kolumbus</td>
<td>6,49</td>
<td>0,078</td>
<td>3,25</td>
<td>3175</td>
<td>2,96</td>
<td>2</td>
</tr>
<tr>
<td><em>Festuca pratensis</em></td>
<td>Patra</td>
<td>13,01</td>
<td>0,050</td>
<td>3,25</td>
<td>5150</td>
<td>2,41</td>
<td>4</td>
</tr>
<tr>
<td><em>Festuca arundinacea</em></td>
<td>Kora</td>
<td>17,45</td>
<td>0,078</td>
<td>2,91</td>
<td>8534</td>
<td>2,80</td>
<td>6</td>
</tr>
<tr>
<td><em>Lolium perenne</em></td>
<td>Handicap</td>
<td>5,36</td>
<td>0,047</td>
<td>2,68</td>
<td>2623</td>
<td>2,16</td>
<td>2</td>
</tr>
<tr>
<td><em>Lolium perenne</em></td>
<td>Korok</td>
<td>11,19</td>
<td>0,057</td>
<td>2,80</td>
<td>5470</td>
<td>2,27</td>
<td>4</td>
</tr>
<tr>
<td><em>Lolium multiflorum</em></td>
<td>Prolog</td>
<td>5,25</td>
<td>0,042</td>
<td>2,62</td>
<td>2567</td>
<td>2,35</td>
<td>2</td>
</tr>
<tr>
<td><em>Lolium multiflorum</em></td>
<td>Lubina</td>
<td>10,99</td>
<td>0,092</td>
<td>2,75</td>
<td>5374</td>
<td>1,96</td>
<td>4</td>
</tr>
</tbody>
</table>

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*

Sorting of individual/groups of chromosomes

Theoretical flow-karyotype

Chromosomes 1, 5, 6
Chromosomes 2, 3, 7
Chromosome 4

*Festuca pratensis*/CV=2.5%
Introgression (substitution) lines

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

\[ \downarrow \]

F1: (2n=3x=21, FpLmLm) x Lm (2n=4x=28)

\[ \downarrow \]

F2: (2n=4x=28, FpLmLmLm) x Lm (2n=4x=28)

\[ \downarrow \]

F3 etc.
Chromosome pairing in substitution lines

Kopecky et al. 2008 Chromosome Res
Chromosome pairing in substitution lines

Kopecky et al. 2008 Chromosome Res
Development of recombinant lines

\[ Fp \]
Distribution of homoeologous recombination in *Festuca/Lolium*
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)
Diversity in hybrids

Individual plants

Bulked samples

Based on 1471 informative DArT markers
Anchoring DArT markers to individual Fp chromosomes

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

<table>
<thead>
<tr>
<th></th>
<th><em>Festuca pratensis</em></th>
<th><em>Lolium multiflorum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre-DArT markers</td>
<td>DArT</td>
</tr>
<tr>
<td>LG1</td>
<td>61</td>
<td>23</td>
</tr>
<tr>
<td>LG2</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>LG3</td>
<td>34</td>
<td>22</td>
</tr>
<tr>
<td>LG4</td>
<td>68</td>
<td>22</td>
</tr>
<tr>
<td>LG5</td>
<td>70</td>
<td>12</td>
</tr>
<tr>
<td>LG6</td>
<td>49</td>
<td>27</td>
</tr>
<tr>
<td>LG7</td>
<td>65</td>
<td>32</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>373</strong></td>
<td><strong>148</strong></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Tolerant</th>
<th>Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freezing</td>
<td>51</td>
<td>45</td>
</tr>
<tr>
<td>Snow mold</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>Rust</td>
<td>71</td>
<td>14</td>
</tr>
</tbody>
</table>
Extreme dry-down
UCR, Riverside CA
early October
(no rain, no irrigation since late July;
temperatures often exceeding 100°F)

F. arundinacea  Festulolium

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)
Acknowledgements

2001-2005
Grant Agency of the Academy of Sciences of the Czech Republic (award no. S5038104): Molecular cytogenetic and cytometric methods for breeding of grasses and trifolium (PI: J. Doležel)

2007-2009
Czech Science Foundation (grant award 521/07/P479): Cytogenetic mapping of genome of meadow fescue (*Festuca pratensis* Huds.) (PI: D. Kopecky)

2007-2010
The National Agency for Agriculture Research (grant award QH71267): The development and use of DArT array for xFestulolium breeding (PI: V. Černoch)

2011-2014
Czech Science Foundation (grant award 501/11/504): Genome interactions in interspecific hybrids xFestulolium (PI: D. Kopecky)
Acknowledgements

Institute of Experimental Botany
Jaroslav Doležel
Jan Bartoš
Pavla Chrystelová
Jarmila Cíhalíková
Mirosława Havránková
Jitka Kopecká
Štěpán Stočes

Diversity Arrays Technology
Canberra, Australia
Andrzej Kilian
Helen Blois
Vanessa Caig

Department of Botany and Plant Sciences
University of California, Riverside, USA
Adam J. Lukaszewski
James H. Baird

Breeding Station Hladké Životice
Vladimír Černoch
Michal Klíma

Agroscope Reckenholz Tanikon Research Station ART, Zurich, Switzerland
Roland Kölliker

Norwegian University of Life Sciences, Ås, Norway
Simen Rød Sandve
Odd Arne Rognli

TEAGASC, Oak Park, Carlow, Ireland
Susanne Barth
Celine Tomaszewski

University of Coimbra, Portugal
João Carlos Mano Loureiro
Silvia Castro-Loureiro
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