

Complete Chloroplast Genome Sequence of Hard Fescue (*Festuca brevipila*)

Yinjie Qiu, Cory Hirsch, Eric Watkins
 Department of Horticultural Science, University of Minnesota
 Department of Plant Pathology, University of Minnesota
 Nov 5, 2018
 Baltimore, MD



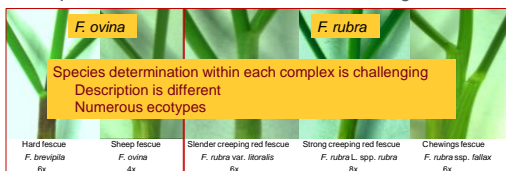
Genome Size Based Species Identification

- Polyploid level isn't necessarily associated with DNA content
 - Genome size can increase or decrease as a response to many factors unrelated to polyploidy
 - Transposon amplification (Hawkins et al., 2006)
 - Repeated deletion of small pieces of DNA (Devos et al., 2002; Woodhouse et al., 2010)

Hawkins, J. S., Kim, H., Nason, J. D., Wing, R. A., & Wendel, J. F. (2006). Differential lineage-specific amplification of transposable elements is responsible for genome size variation in *Sorghum*. *Genome research*, 16(10), 1262-1267.
Devos, K. M., Brugg, J. K., & Bennett, J. L. (2002). Genome size reduction through illegitimate recombination counteracts genome expansion in *Arabidopsis*. *Genome research*, 12(7), 1075-1079.
Woodhouse, M. R., Schmalz, J. C., Pedersen, B. S., Lyons, E., Lisch, D., Subramaniam, S., & Freeling, M. (2010). Following tetraploidy in maize, a short deletion mechanism generated genes preferentially from one of the two homoeologs. *PLoS biology*, 8(5), e1000463.

Fine Fescue (*Festuca* spp.)

- Shade, drought tolerant, often seeded as mixture
- Require little to no additional fertilizer or irrigation



Can We Develop a Better Method for Species Identification?

Fine Fescue Species Determination

- Laser Flow Cytometry (Huff, 1998)
 - Measure physical and chemical characteristics of a cell
 - DNA content was associated with polyploidy for species classification

Species	Mean DNA content (pg)	Range (Low)	Range (High)
Hard fescue (HF) (6x)	12.55 *	11.41	13.54
Sheep fescue (SHF) (4x)	9.11	NA	NA
Slender creeping red fescue (SLFR) (6x)	10.89	10.45	11.16
Strong creeping red fescue (STRF) (8x)	14.27	12.54	15.53
Chewings fescue (CHF) (6x)	11.91	11.24	12.37

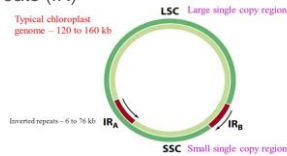
DNA Sequence for Species Identification

- Nucleus gene sequence (Baldwin et al., 1995)
 - Biparentally inherited, large variation
 - Challenging in polyploidy species
- Chloroplast gene sequence (Martin et al., 2002)
 - Maternally inherited, highly conserved within species
- Mitochondrial gene sequence (Li et al., 2001)

Baldwin, Bruce G., et al. "The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny." *Annals of the Missouri Botanical Garden* 1990: 247-277.
Martin, William, et al. "Evolutionary analysis of *Arabidopsis*, *Cardoideae*, and *Chenopodiaceae* genomes reveals distinct phylogeny and thousands of cytoskeletal genes in the nucleus." *Proceedings of the National Academy of Sciences* 10 (2003): 12246-12251.
Li, Ming, et al. "An information-based sequence distance and its application to whole mitochondrial genome phylogeny." *Bioinformatics* 17.2 (2001): 149-154.

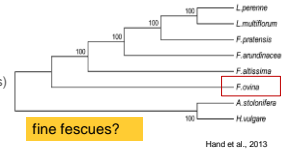
Chloroplast Genome Structure

- Circular DNA
- High sequence conservation
- Two identical inverted repeats (IR)
- Large single-copy (LSC)
- Small single-copy (SSC)



Phylogeny of *Festuca-Lolium* Complex

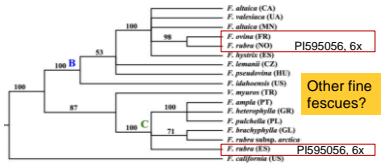
- Phylogeny reconstruction using plastome sequence
 - *F. ovina* (diploid)
 - *F. pratensis* (Meadow fescue)
 - *F. altissima* (Wood fescue)
 - *L. multiflorum* (Italian ryegrass)
 - *L. perenne* (Perennial ryegrass)
 - *F. arundinacea* (Tall fescue)
 - *A. stolonifera* (Creeping bentgrass)
 - *H. vulgare* (Barley)



Hand, M. L., Spangenberg, G. C., Farber, J. W., & Cogan, N. O. (2013). Plastome sequence determination and comparative analysis for members of the Lolium-Festuca grass species complex. *D2: Genes, Genomes, Genetics*, 63-112.

Phylogeny of *Festuca-Lolium* Complex

- Chloroplast gene based fine fescue classification
 - *trnQ-rps16*
 - *trnH-psbA*
 - *rbcL*



Chang, Yiqian, et al. "Phylogenetic relationships in the *Festuca-Lolium* complex (Liliaceae: Poaceae): new insights from chloroplast sequences." *Frontiers in Ecology and Evolution* 4 (2016): 86.

Objectives:

1. Use Chloroplast Genome Sequencing to Provide Phylogeny Relationships of Five Fine Fescues
2. Use Sequence Information to Develop Markers for Species Identification

Chloroplast Genome for Phylogeny

- Genome contains more genes, provide more information
 - High resolution plant phylogenies (Parks et al., 2009)
- Highly conserved
 - gene order
 - gene content (Raubeson and Jansen 2005)
- Exhibit a lower substitution rate than nuclear DNA (Wolfe et al., 1987)

• Parks, M., Cronn, R., & Lister, A. (2009). Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biology*, 7(1), 84.

• Raubeson, L.A. Jansen, R.K. Henry. R2 Chloroplast genomes of plants: Diversity and evolution of plants—genotypic and phenotypic variation in higher plants 2005 Wallingford (UK): CAB International, 45-66.

• Wolfe, K. H., Li, W. H., & Sharp, P. M. (1987). Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences*, 84(24), 1054-1058.

Material and Sequencing Methods

Plant material

Hard fescue	Sheep fescue	Strong creeping red fescue	Slender creeping red fescue	Chewings fescue
Beacon	Quatro	Navigator II	Shoreline	Treasure II

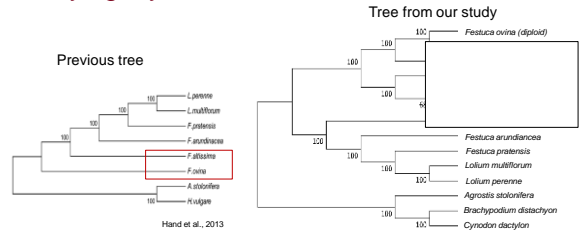
- Whole genome sequencing was done via HiSeq4000 platform, 150 bp PE mode
- 2G raw reads were generated for each samples

Chloroplast Genome Assembly

- NovoPlasty assembler (V 2.7.2) was used to generate circular genome using *F. ovina* genome (JX871940) as reference
- Chloroplast genomes from other grass species were used for assembly validation

Dierckx, N., Mardijn, P., & Smit, G. (2016). NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic acids research*, 44(1), e114-18.

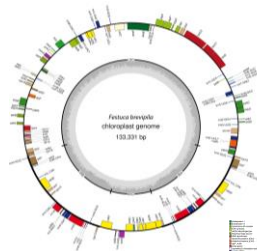
Chloroplast Genome Based Phylogeny Tree Reconstruction



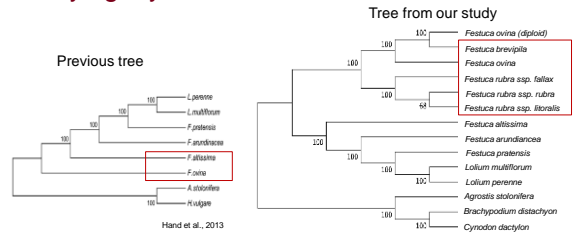
Hand, M. L., Spangenberg, G. C., Farber, J. W., & Cogan, N. D. (2013). Plastome sequence determination and comparative analysis for members of the Lolium-Festuca grass species complex. *D2: Grasses, Genomes, Genetics*, g3-112.

Hard Fescue Chloroplast Genome

- 133,331 bp
- 85 protein coding genes
- 38 predicted tRNA genes
 - LSC 25 tRNAs
 - IR 12 tRNAs
 - SSC 1 tRNA



Chloroplast Genome Based Phylogeny Tree Reconstruction



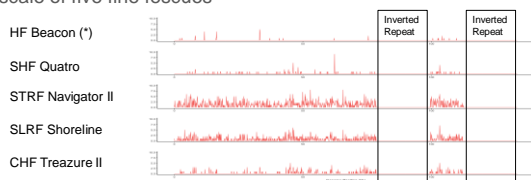
Hand, M. L., Spangenberg, G. C., Farber, J. W., & Cogan, N. D. (2013). Plastome sequence determination and comparative analysis for members of the Lolium-Festuca grass species complex. *D2: Grasses, Genomes, Genetics*, g3-112.

Comparative Genomics of Fine Fescue Species

Characteristics	<i>Festuca brevipilis</i> Beacon	<i>Festuca ovina</i> Quatro	<i>Festuca rubra</i> ssp. <i>fallax</i> Treasure II	<i>Festuca rubra</i> ssp. <i>iborralis</i> Shoreline	<i>Festuca rubra</i> ssp. <i>rubra</i> Navigator II
Size (bp)	133,331	133,508	133,842	133,814	133,804
LSC	78,462	78,632	78,882	78,909	78,888
SSC	12,393	12,400	12,452	12,435	12,446
IRs	42,476	42,476	42,508	42,470	42,470
Ratio of LSC (%)	58.85%	58.90%	58.94%	58.97%	58.96%
Ratio of SSC (%)	9.29%	9.29%	9.30%	9.29%	9.30%
Ratio of IRs (%)	31.86%	31.82%	31.76%	31.74%	31.74%

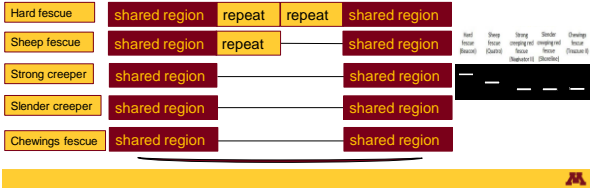
Chloroplast Genome Sequence Variation for Marker Development

Single Nucleotide Polymorphism distribution at genome scale of five fine fescues



Chloroplast Genome Structural Variation for Marker Development

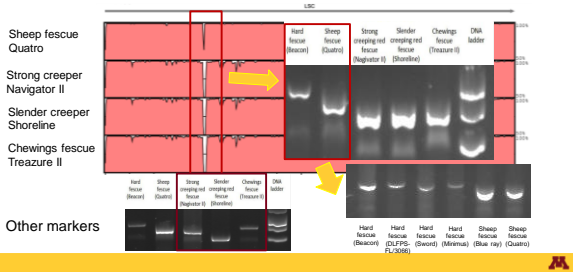
- Tandem repeat is evolutionarily consistent
 - Consensus sequence (repeat) copy number variation



Summary and Future Research

- We present the phylogeny relationship of fine fescue species within *Festuca – Lolium* complex through DNA sequencing
- We developed DNA markers for fine fescue species identification
- This knowledge will benefit future germplasm collection and screening
- Species specific markers could potentially help us study the dynamics of fine fescue mixtures

Tandem Repeat Marker

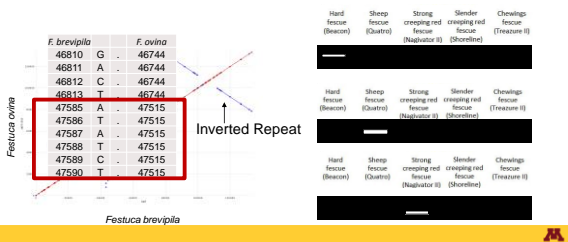


Acknowledgements

- Drs. Adrian Hegeman, Angela Orshinsky, Laura Shannon
- Turfgrass research group at University of Minnesota
- Minnesota Supercomputing Institute
- Drs. Peter Morrell and Ya Yang at University of Minnesota
- Funding:

National Institute of Food and Agriculture,
 U.S. Department of Agriculture,
 Specialty Crops Research Initiative under award number 2017-51181-27222

Development of Species Specific Markers



QUESTIONS?



Whole Chloroplast Genome Alignment

