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Overview

Meadow fescue (*Festuca pratensis* L.) is a perennial, cool season grass commonly found in cool pastures throughout the world. A notable exception is in the United States, where it has seen relatively little use despite having been present in this country since the mid-18th century, and currently growing in pastures throughout the Driftless Area. Meadow fescue provides forage quality nearly equivalent to ryegrass (*Lolium* spp.), while being able to persist under greater cold and drought stresses. These attributes have led to an increased interest in incorporation of meadow fescue as a component in pasture species assemblages in the North Central USA, which in turn has prompted this work. Meadow fescue provides an excellent case study for the process of attaining a more complete understanding of an organism's biology in the early stages of its adoption.

There are three main considerations I felt needed to be addressed in order to manage the promotion and expansion of meadow fescue as responsibly as possible. First is whether the existing population structure of meadow fescue in the Driftless Area possesses some intrinsic value, such as adaptation to local environmental conditions. Such value might be eroded by introduction of poorly adapted cultivars or increased movement of propagules from other sites. Second is how well suited meadow fescue is to the general environment of the Driftless Area. If meadow fescue can only persist as a substantial pasture component within a narrow environmental envelope, we would need to define that space to know whether meadow fescue could become widespread in the region, either through intentional establishment or natural range expansion. Finally, we should assess whether meadow fescue shows indications of potential invasiveness, such as evidence of rapid, recent range expansion or a capacity for competitive exclusion. Other species, tall fescue and reed

canarygrass among them, have been introduced as potential forages but have expanded into areas where they are undesirable. Given this history, it is critical to establish whether meadow fescue carries a substantial risk for such expansion. I conducted three projects whose interpretations would overlap address these concerns: a genetic study of meadow fescue sites from across the Driftless Area, a field survey of the relative abundance of meadow fescue and the growing conditions at these same collection sites, and a greenhouse experiment testing how nitrogen addition and defoliation height influence the outcome of competition between meadow fescue and frequent companion species.

The results, analysis, and interpretation of the genetic study form Chapter 1 of this thesis. The purpose of this component was to study genetic structure and diversity, both within sites and across the region. The population structure would provide indications of introduction history in the region and should contain evidence of population expansion, as well as estimates of the dispersal ability of genetic material among sites. Recent expansion history and dispersal ability would provide evidence of past or potential invasiveness. Isolation and differentiation among sites, as well as the distribution of diversity present within sites, would indicate the potential for useful functional diversity and local adaptation. Appendix 1 provides details about diversity and connectivity metrics for individual sites, which are presented in aggregated form the chapter. Expanded information about the study's SSR markers and their scoring is presented in Appendix 2, while specifics on the tests used to identify the different chloroplast haplotypes are described in Appendix 3.

The second chapter contains the results of the greenhouse competition experiment. This experiment was intended to address the lack of consideration given in the field study to the effect of different management styles on meadow fescue's ability to establish and

compete with other pasture grasses. By testing how different management styles would affect the outcome of competition between meadow fescue and other grasses, we would gain information on whether management could be altered to improve, or inhibit, meadow fescue establishment. The analysis of competition dynamics, particularly evidence of displacement, would serve to evaluate whether meadow fescue could exclude other species, further evaluating its potential risk as an invasive species.

The methods and results from the field study are presented in Appendix 4. The intent of this study was to establish the range of conditions under which meadow fescue has naturalized in the Driftless Area, as well as to characterize the prominence of meadow fescue in the local species assemblage. The abundance of meadow fescue could be modeled as a function of factors in the environment, leading to a determination of which environmental factors are linked to meadow fescue abundance, and how the interaction of these factors is perceived by meadow fescue. While the structure of the study made it difficult to draw meaningful conclusions from the data, this survey still provides a useful description of the range of habitats in which meadow fescue has established and the plant communities in which it can be found. Thus, the data are summarized here with minimal analysis and no interpretation.

Chapter 1.

Multiple introductions of meadow fescue (*Festuca pratensis* Huds.) into Driftless Area inferred from spatial genetic structure

Introduction

The combination of environmental change and increased human-mediated connectivity has dramatically altered species distribution, with some species expanding into new habitats while others face extinction in their native ranges (Franco *et al.*, 2006; Kowarik, 2003). The European cool-season pasture grass meadow fescue [*Festuca pratensis* Huds.; *Schedonorus pratensis* (Huds.) P. Beauv.] is among the species experiencing both scenarios. In recent decades, meadow fescue presence has decreased substantially throughout lower latitude and elevation areas of its European range (Carlen *et al.*, 2002; Peter-Schmid *et al.*, 2008b). European field studies have found that it fares poorly in direct competition with common pasture grasses including orchardgrass (Dactilys glomerata L.Carlen *et al.*, 1999) and timothy (*Phleum pratense* L., Jorgensen, Nosberger, 1994), and generally performs poorly under intensive management (Peter-Schmid *et al.*, 2008a; Zimmermann, Nosberger, 1999). In contrast, volunteer populations of meadow fescue are being identified in pastures throughout the Driftless Area in Wisconsin and neighboring states (Casler *et al.*, 2008), frequently becoming a major component of local species assemblages (Appendix 4).

A change in meadow fescue's range could have considerable economic ramifications. Meadow fescue is among the few grasses with high forage quality that also exhibit substantial winter hardiness, making it a very important species for pastures in cooler regions of the world (Carlen *et al.*, 1999; Niemelainen *et al.*, 2001). Diminished abundance

throughout its historic range is accompanied by a noticeable decrease in genetic diversity compared to orchardgrass and ryegrass (Lolium perenne L., Kolliker *et al.*, 1999; Peter-Schmid *et al.*, 2008a). Maintenance of genetic diversity is essential both for future improvement of important agronomic characteristics (Loos, 1993), and for the continued viability of the entire species (Ellstrand, Elam, 1993). Concerns over erosion of diversity in meadow fescue have prompted calls to evaluate pastures and grasslands in Europe as potential reservoirs of genetic diversity (Fjellheim, Rognli, 2005; Peter-Schmid *et al.*, 2008b). Given the relatively greater ecological success of meadow fescue in the Driftless Area, populations from this region may also represent useful sources of genetic material.

Beyond serving as diversity reservoirs for the species at large, Driftless Area meadow fescue has the potential to become a valuable component of local agricultural, particularly in the rotational grazing management systems that are becoming increasingly popular in this region (Casler *et al.*, 1998). As a greater range of ecological services are expected of agroecosystems, maintenance of biodiversity has become important both as a means of enhancing these services and as a service in its own right (Sanderson *et al.*, 2004). Proper species selection is essential to maintenance of sustainable pasture assemblages (Kemp *et al.*, 2000), and locally adapted meadow fescue ecotypes are better able to establish in mixed swards (Makela, Kousa, 2009). Thus, estimation of the degree to which naturalized meadow fescue populations have adapted to local growing environments in the Driftless Area would be helpful in evaluating the potential of this grass for incorporation into the region's agoecosystems.

In addition to its potential economic value, meadow fescue in the Driftless area provides an interesting system with which to study how range expansion affects genetic

diversity and adaptation. The partitioning of genetic variability and retention of diversity despite small founder populations is similar between meadow fescue and many tree species (Austerlitz *et al.*, 2000; Fjellheim, Rognli, 2005), making this system a possible faster-paced analogue for testing generalizability of dispersal and connectivity models for trees. Additionally, as native ranges shift away from historic selective conditions, it may become increasingly common for species to thrive in novel habitat while becoming threatened in their traditional range. Meadow fescue could serve as an interesting case study of the role populations in an expanded range can play in maintaining the viability of the species in its native habitat.

This study consists of three aspects. First, we determined the population structure of meadow fescue in the Driftless Region, looking for evidence of separate introductions or local adaptation to inform future breeding and conservation efforts. Second, we surveyed levels of genetic diversity, both for the region as a whole and within the context of the population structure. Finally, we evaluated gene flow and population turnover at the regional and local levels

Materials and methods

Study area and plant materials

The Driftless Area is the unglaciated region along the upper Missisippi River, primarily distinguished by a highly eroded landscape with rolling hills and substantial forest cover (Knutson *et al.,* 2004). Agriculture, particularly field crops and pasture, dominates the landscape with Eurasian cool-season grasses as the dominant pasture taxa (Renfrew, Ribic, 2002). Meadow fescue has been identified in over 200 farms with meadow fescue, primarily across southwest Wisconsin. This study includes plants from 48 farms spread throughout

most of this range, with a mean separation between farms of 7.23 km (SD 6.93). Plants from 71 collection sites were included in the study, with 21 farms having multiple collections (Table 1). The mean distance between same-farm sites was 0.37 km (SD 0.46).Where possible we emphasized paired collections from shaded and unshaded sites within farms, finding 14 such pairings. Sites were further grouped by slope, use as pasture, and adjacency to trees (Table 1).

Plants were sampled in a spoke and wheel pattern. A plant in the center of a relatively dense patch was selected as the center and plants were sampled radially from there every 45˚at 0.3, 0.6, 1.2, and 2.4 m intervals, for a total of 33 collections per site. Care was taken to collect only physically connected tillers. DNA was extracted from plant leaves following the protocol of Storchova *et al.* (2000).

Table 1. Location, site characteristics, and dominant subpopulation of study sites

Site Number	Site Name	Lat.	Long.	Shading	Slope	Pasture	Tree adj.	Dominant subpop.
18	WI-CAE-LF	42˚ 53'	90˚ 15'	Sun	Flat	Yes	Yes	IA
19	WI-CAE-DF	42˚ 53'	90˚ 15'	Shade	Flat	Yes	Yes	IA
20	WI-15A-LF	42˚ 49'	90˚ 16'	Sun	Flat	Yes	No	IA
21	WI-15A-LG	42˚ 49'	90˚ 16'	Sun	Gully	Yes	No	IA*
22	WI-EDW-LH	42˚ 59'	90˚ 21'	Sun	Flat	Yes	No	MX
23	WI-EDW-DF	42˚ 59'	90˚ 21'	Shade	Flat	Yes	Yes	MX
24	WI-Y19-LH	42˚ 57'	90˚ 03'	Sun	Hill	Yes	Yes	MX
25	WI-Y19-LH	42˚ 57'	90˚ 04'	Sun	Hill	Yes	Yes	MX
26	WI-YLA-LF	42˚ 55'	90˚ 05'	Sun	Flat	Yes	Yes	WI
27	WL-FNP-LF	42˚ 48'	89˚ 56'	Sun	Flat	No	Yes	WI*
28	WL-FNP-LH	42˚ 48'	89˚ 56'	Sun	Hill	Yes	Yes	WI
30	WL-WOO-LF	42˚ 42'	90˚ 04'	Sun	Flat	Yes	Yes	IA*
31	WI-CAT-DG	42˚ 59'	90˚ 18'	Shade	Gully	Yes	Yes	IA
33	WI-GRO-LF	42˚ 56'	90˚ 19'	Sun	Flat	Yes	No	IA
34	WI-GRO-LH	42˚ 56'	90˚ 19'	Sun	Hill	Yes	No	IA*
35	WG-ESC-LF	42˚ 54'	90˚ 34'	Sun	Flat	Yes	Yes	WI
36	WG-ESC-LH	42˚ 54'	90˚ 34'	Sun	Hill	Yes	Yes	MX
37	WG-LIM-DF	42˚ 58'	90˚ 39'	Shade	Flat	Yes	Yes	MX

Table 1 cont.

38	WG-LIM-LF	42˚ 58'	90˚ 39'	Sun	Flat	Yes	No	GR
39	WL-TR1-DF	42˚ 43'	90˚ 18'	Shade	Flat	Yes	Yes	WI
40	WL-TR1-LH	42˚ 42'	90˚ 18'	Sun	Hill	Yes	No	WI
41	WI-OP1-LF	42˚ 50'	90˚ 14'	Sun	Flat	Yes	Yes	MX
42	WI-OP1-DF	42˚ 50'	90˚ 14'	Shade	Flat	Yes	Yes	IA*
43	WI-HEJ-DH	42˚ 52'	90˚ 19'	Shade	Hill	No	Yes	IA
44	WG-DRI-DF	42˚ 53'	90˚ 31'	Shade	Flat	Yes	Yes	MX
46	WG-DRI-LF	42˚ 54'	90˚ 31'	Sun	Flat	Yes	Yes	MX
47	WG-DRI-LH	42˚ 54'	90˚ 31'	Sun	Hill	Yes	Yes	MX
48	WG-BAG-LF	42˚ 53'	90˚ 49'	Sun	Flat	Yes	No	MX
49	WI-CAE-DH	42˚ 53'	90˚ 15'	Shade	Hill	Yes	Yes	IA
50	WI-JOW-LF	42˚ 57'	90˚ 18'	Sun	Flat	Yes	No	IA
51	WI-FAS-DF	42˚ 57'	90˚ 15'	Shade	Flat	Yes	Yes	IA*
52	WI-FAS-LF	42˚ 58'	90˚ 15'	Sun	Flat	Yes	No	IA
53	WI-CHS-DH	42˚ 58'	90˚ 10'	Shade	Hill	Yes	Yes	MX
54	WI-CHS-LF	42˚ 57'	90˚ 10'	Sun	Flat	Yes	No	MX
55	WI-ENL-LF	42˚ 54'	90˚ 23'	Sun	Flat	Yes	Yes	IA
56	WI-IG8-LF	42˚ 56'	90˚ 25'	Sun	Flat	Yes	No	IA*
57	WI-BDR-LF	42˚ 57'	90˚ 22'	Sun	Flat	Yes	No	IA*
58	WG-SHR-DH	42˚ 53'	90˚ 36'	Shade	Hill	Yes	Yes	GR
59	WG-SHR-LH	42˚ 53'	90˚ 36'	Sun	Hill	Yes	Yes	GR
60	WG-FWE-LH	42˚ 57'	90˚ 38'	Sun	Hill	Yes	No	MX
61	WG-LRH-LF	42˚ 53'	90˚ 38'	Sun	Flat	Yes	No	GR
62	WG-LRH-DH	42˚ 53'	90˚ 38'	Shade	Hill	Yes	Yes	GR
63	WL-BUO-DH	42˚ 46'	90˚ 13'	Shade	Hill	No	Yes	IA
64	WG-REB-LH	42˚ 44'	90˚ 39'	Sun	Hill	Yes	Yes	GR
65	WG-REB-LF	42˚ 44'	90˚ 39'	Sun	Flat	Yes	Yes	GR
71	WG-RON-DF	42˚ 45'	91˚ 01'	Shade	Flat	Yes	Yes	MX
73	WG-RON-LF	42˚ 45'	91˚ 02'	Sun	Flat	Yes	No	WI
74	WL-KAM-LF	42˚ 40'	90˚ 07'	Sun	Flat	Yes	Yes	MX
75	WL-DUH-DH	42˚ 34'	90˚ 04'	Shade	Hill	Yes	Yes	WI
76	WG-CEN-LF	42˚ 33'	90˚ 29'	Sun	Flat	Yes	No	WI
77	WV-BAG-LH	43˚ 42'	91˚ 00'	Sun	Hill	Yes	Yes	WI*
78	WV-BAG-DH	43˚ 42'	91˚ 00'	Shade	Hill	Yes	Yes	WI
79	WC-POB-LF	43˚ 45'	91˚ 11'	Sun	Flat	Yes	No	LX
80	WG-MUS-DF	42˚ 48'	90˚ 56'	Shade	Flat	Yes	Yes	WI
81	WG-61C-LF	42˚ 42'	90˚ 42'	Sun	Flat	Yes	Yes	GR
82	WG-SBK-LF	42˚ 48'	90˚ 44'	Sun	Flat	Yes	Yes	MX
83	WC-OAT-LF	43˚ 49'	91˚ 07'	Sun	Flat	Yes	Yes	MX

Table 1 cont.

85	WV-PIG-LH	43˚ 43'	90˚ 59'	Sun	Hill	Yes	Yes	LX
86	WR-TWU-LF	43˚ 17'	90˚ 39'	Sun	Flat	Yes	Yes	MX
100	IA-VIL-DF	43˚ 18'	91˚ 22'	Shade	Flat	Yes	Yes	GR
101	IA-EBD-DF	43˚ 22'	91˚ 25'	Shade	Flat	No	Yes	GR
103	WG-HUV-LF	42˚ 47'	90˚ 38'	Sun	Flat	No	Yes	MX
104	WG-PIK-LF	42˚ 44'	90˚ 45'	Sun	Flat	Yes	Yes	WI
105	IC-LIF-LF	42˚ 41'	91˚ 25'	Sun	Flat	Yes	Yes	WI
106	MH-33S-LF	43˚ 31'	91˚ 18'	Sun	Flat	Yes	Yes	MX
107	MH-33S-DH	43˚ 31'	91˚ 18'	Shade	Hill	Yes	Yes	MX
108	MH-241-LF	43˚ 31'	91˚ 30'	Sun	Flat	Yes	Yes	WI
109	MH-241-DH	43˚ 31'	91˚ 30'	Shade	Hill	Yes	Yes	WI
110	MH-267-LF	43˚ 33'	91˚ 17'	Sun	Flat	No	No	LX
111	MH-267-LH	43˚ 33'	91˚ 17'	Sun	Hill	Yes	Yes	LX
112	WI-EBU-LF	42˚ 53'	90˚ 14'	Sun	Flat	Yes	Yes	IA

Latitude and longitude are in degrees and minutes north and west respectively. Pasture and tree adjacency indicate whether sampling site was located in a field used as a pasture or adjacent to trees, respectively.

* sites had major subpopulation assignment < 0.75 (see text for details).

Molecular protocols

All PCR amplification used the JumpStart amplification system (Sigma) following manufacturer recommendations, with Betaine (Sigma) added to a final concentration of 0.50 µM for SSR amplification only. Simple-sequence repeat (SSR) markers were assayed with primers variously designed from *Lollium* sp. or *Festuca arundinacea* (outlined in Table 2 and in greater detail in Appendix 2). We identified 20 polymorphic loci with no null alleles, many of which were described in Peter-Schmid *et al*. (2008a) with a combined 191 alleles in the study population (Table 2). SSR amplification was done with 45 cycles with annealing at 50 ˚C for 60 s and extension at 72 ˚C for 120 s. SSRs were amplified and separated in multiplex (described in Appendix 2). Capillary electrophoresis was conducted on Applied

Biosystems 3100 and 3700 capillary sequencers with the GS 500 ROX size standard. Visualization and scoring were done with GeneMarker v1.75 (SoftGenetics).

The MFhapA1 marker assay consisted of amplification of a segment in the trnS-psbD region containing a T → A substitution characteristic of the A haplotype. Digest with the *PsiI* enzyme yields a distinct banding pattern in A haplotype individuals, allowing visual scoring by gel electrophoresis. The MFhapC marker assays the 6-bp insertion that characterizes the C haplotype, as well as the 1-bp insertion characterizing the D haplotype (Table 3) by capillary electrophoresis of a segment of the trnT2-rps4 region. As no positive markers exist for the B haplotype, it is defined by the absence of the other haplotype markers. Methods for both assays are described in detail in Appendix 3.

Statistical Techniques

Estimates of genetic diversity and genetic distance, spatial autocorrelation, and AMOVA were all conducted with GENALEX v 6.1 (Peakall, Smouse, 2006). Allelic diversity, unbiased expectected heterozygosity (UHe), polymorphism, and Nei's genetic distance (Nei, 1978) were calculated using the Frequency option for codominant data, using collection sites as populations. Partitioning of genetic variance was done with the AMOVA option for codominant genotypic data with specialized permutation within regions (Excoffier *et al.*, 1992). Within-site spatial structure was estimated for each site using the spatial autocorrelation method for single populations; subsequently sites with similar structural patterns were analyzed jointly using the multiple populations option.

Population structure was inferred by cluster analysis with the program STRUCTURE v 2.3.1, using an admixture model with correlated allele frequencies (Pritchard *et al.*, 2000). Initial range determination consisted of 3 independent runs with 10,000 burn-ins and 100,000

MCMC iterations for $K = 2$ to $K = 7$. Maximum likelihood was occurred at $K = 6$, though this model represented very limited improvement over simpler models. We subsequently conducted 6 independent runs with 100,000 burn-ins and 1,000,000 MCMC for $K = 3$ to $K = 6$ using the resources of the Computational Biology Service Unit from Cornell University (partially funded by Microsoft Corporation). Selection of the $K = 4$ model was based on recommendations in Pritchard *et al.* (2009), as this model showed a marked improvement in likelihood over $K = 3$, while more complicated models had much smaller increases in likelihood and did not substantially alter the structure of the results. Assignment proportions for individuals and sites, as well as allele frequencies for populations, were averaged across the six runs.

Table 2. Allelic diversity and heterozygosity for SSRs used in this study.

Marker	Total Alleles	Rare Alleles	Alleles per site	Ho	Uhe	Reference
B1A8	23	10	8.77	0.791	0.797	Lauvergeat *et al.*, 2005
DLF008	7	2	3.96	0.580	0.589	
LpSSR112	4	1	2.73	0.347	0.360	(Jensen *et al.*, 2005)
Uni001	35	18	10.59	0.746	0.774	(Jensen *et al.*, 2005)
LM15	7	4	2.75	0.505	0.506	
LM26	16	6	6.13	0.601	0.663	(Studer *et al.*, 2006)
LMgSSR 02-05A	6	2	3.00	0.567	0.535	Hirata *et al.*, 2006
M4213	4	2	1.27	0.031	0.031	
PR3	12	5	5.96	0.706	0.723	(Kubik *et al*., 2001)
NFA022	2	0	1.99	0.418	0.416	
NFA027	15	9	5.77	0.751	0.724	(Saha *et al.*, 2004)
NFA032	2	0	1.28	0.032	0.031	(Saha *et al.*, 2004)
NFA041	5	0	3.63	0.545	0.565	(Saha *et al.*, 2004)
NFA048	3	1	2.01	0.468	0.457	(Saha *et al.*, 2004)
NFA059	6	3	3.61	0.547	0.529	(Saha *et al.*, 2004)
NFA060	4	1	1.83	0.091	0.085	(Saha *et al.*, 2004)
NFA071	11	4	5.32	0.693	0.700	(Saha *et al.*, 2004)
NFA073	2	0	2.00	0.428	0.414	(Saha *et al.*, 2004)
NFA099	23	15	6.76	0.701	0.679	(Saha *et al.*, 2004)
NFA136	4	1	1.42	0.046	0.049	(Saha *et al.*, 2004)

Alleles are considered rare if their overall frequency in the population is below 0.01. Alleles per site, observed heterozygosity (Ho), and unbiased expected heterozygosity (UHe) are arithmetic means across sites.

Table 3. Chloroplast polymorphisms defining meadow fescue haplotypes

Marker	Chloroplast region	Polymorphism	Indicated haplotype
MFhapA1	trnS-psbD	CAAGCAGGTTAT**T**TATAAAAAAAAAG*	A
		CAAGCAGGTTAT**A**TATAAAAAAAAAG*	B/C/D
MFhapC	trnT2-rps4	ACCTATACCT**CAGTCT**TCCGAGAAAG*	C
		ACCTATACCT------TCCGAGAAAG*	A/B/D
MFhapD	trnT2-rps4	AACAAAAAAAAA-GAGTCAAATCAAA	D
		AACAAAAAAAAA**A**GAGTCAAATCAAA	A/B/C

* previously described in (Fjellheim *et al.*, 2006).

Results

Population structure and subpopulations

Results from the STRUCTURE program indicated the data could be most parsimoniously explained by four subpopulations. From this model, we initially categorized sites as dominated by a subpopulation with a membership proportion ≥ 0.75 if all other subpopulation memberships were < 0.20. In addition, we identified 9 sites whose primary membership was < 0.75 but at least 0.63, no secondary membership larger than 0.18, suggesting they were still substantially dominated by a single subpopulation. We conducted separate AMOVAs for each population to evaluate whether distinction between strong and weak dominance had explanatory power. Among sites with a major IA component, this grouping explained 0.8% of the variability, compared to 5.3% explained by site differences within groups; the WI groups explained no variability while having 15.6% of the variability between sites (Table 4). While the IA grouping was statistically significant ($p = 0.001$), the variance explained by this grouping was judged too small to justify analyzing these samples as a discreet entity. As a result, 69% of the sites in our study were considered dominated by one of the four subpopulations (Table 5).

Table 4. AMOVAs testing validity of grouping by strength of dominance in two subpopulations

Subpopulation	Source of variation	df	Variance component	Variance (%)
IA	Among dominance groups	1	0.08*	0.8%
	Among sites within groups	17	0.57*	5.3%
	Within sites	446	10.16*	94.0%
WI	Among dominance groups	1	0.00	0.0%
	Among sites within groups	14	1.64*	15.6%
	Within sites	326	8.89*	84.4%

* significant at p = 0.001 (999 random permutations across the entire population)

Sites dominated by three of the subpopulations had clear geographic distributions, from which we derived their names (Figure 1, solid symbols). The IA and GR subpopulations were located primarily in Wiscoinsin's Iowa and Grant counties, respectively, while the LX subpopulation was in the area around La Crosse, Wisconsin (Figure 1). The WI subpopulation was prevalent along the southern and western edges of the study area, but could be found throughout the range (Figure 1, crosses). Sites dominated by the WI subpopulation were both significantly more dispersed, as measured by mean distance among WI-dominated sites, and more isolated, measured by distance to the nearest site with the same subpopulation (Table 5).

Table 5. Mean values for indicators of connectivity and diversity in dominated and admixed sites

Dominant subpop.	Sites	Rare Alleles	Alleles per locus	UHe	Polymorphic loci	Nearest neighbor (km)	Mean distance (km)
IA	19	0.36 (0.03)	4.55 (0.08)*	0.504 (0.004)*	0.939 (0.011)	4.1 (0.6)*	12.5 (1.0)*
GR	10	0.36 (0.03)	4.34 (0.17)*	0.503 (0.006)*	0.855 (0.017) †	5.9 (0.8)*	40.3 (5.8)*†
LX	4	0.35 (0.11)	4.22 (0.24)	0.498 (0.017)*	0.925 (0.014)	20.3 (2.2) †	25.9 (1.5)*
WI	16	0.22 (0.06)	3.33 (0.13) †	0.427 (0.008) †	0.819 (0.018) †	26.2 (3.1) †	80.5 (4.9) †
MX	22	0.27 (0.03)	3.95 (0.08)*†	0.489 (0.004)*	0.857 (0.010) †		

Standard deviations are in parentheses. The nearest neighbor and mean distance are Euclidian distances to farms with the same dominant subpopulation and exclude distances between sites on the same farm. * significantly different from the WI value, † significantly different from the IA value, both $p < 0.05$ by Tukey-Kramer test.

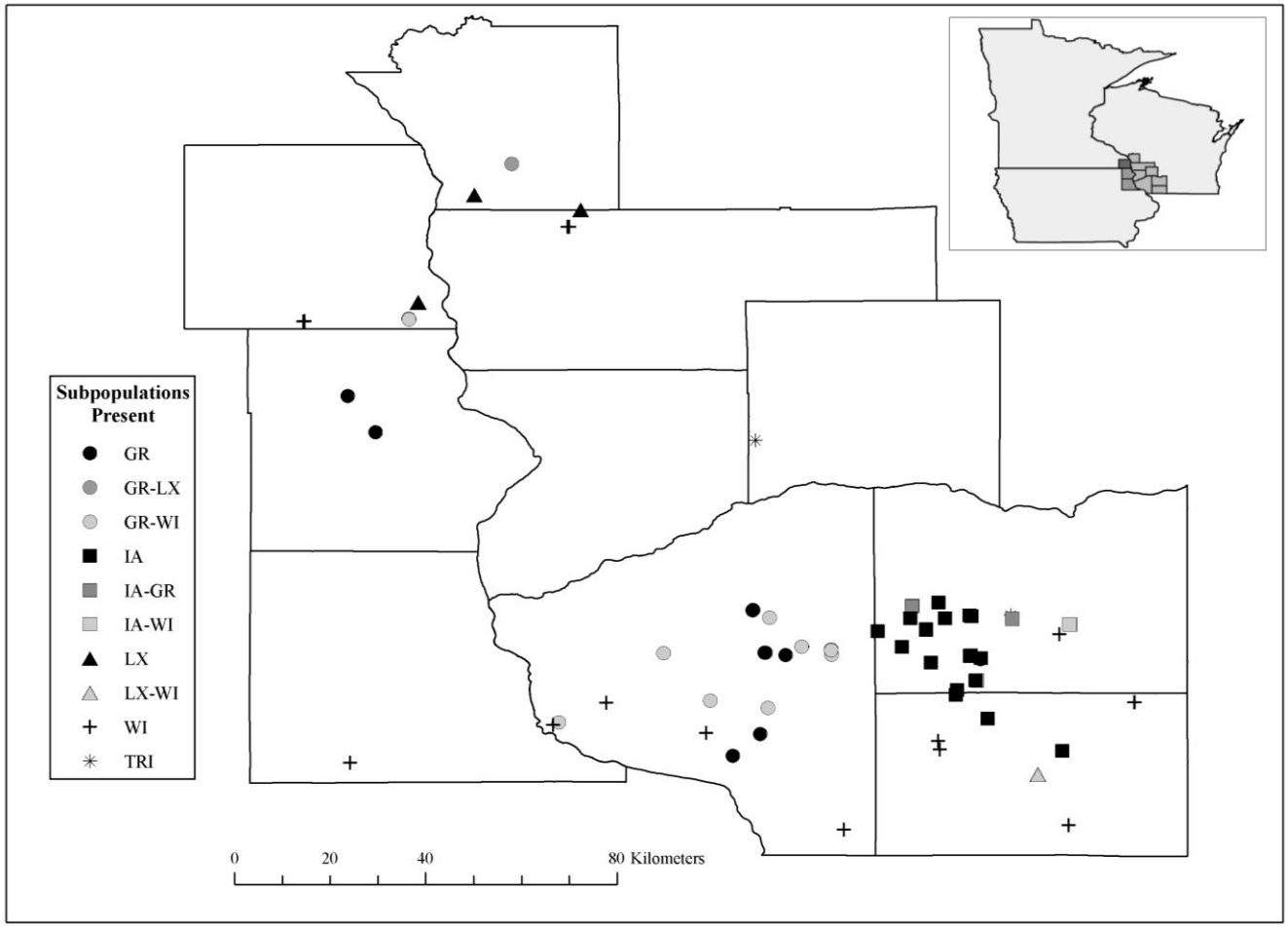

Figure 1. Distribution of meadow fescue subpopulations across the Driftless Area.

Sites dominated by a single subpopulation (see text for assignment criteria) are denoted by solid symbols, while admixtures are gray or white, with the symbol corresponding to the component with the most proximal range.

The remaining sites were considered admixtures of all subpopulations with a > 0.20 membership assignment, with 20 sites having 2 significant members and 2 sites with 3. We observed every possible subpopulation pairing except between IA and LX, which also did not coincide in either of the three-component admixtures. The paired admixtures tended to occur in the overlap of their components' ranges (Figure 1). We considered all admixed sites a single group (MX) for comparison purposes to sites dominated by single subpopulations (Table 5).

Individual plants were assigned to subpopulations following the same convention as with sites, although only the strongest population assignment was considered. We assigned 1132 individuals (68.6%) to specific subpopulations. Among dominated sites, this percentage increased to 77.5%, with only 12 individuals (1.0%) assigned to a population other than the dominant one at their location. Furthermore, only 73 individuals (6.2%) in dominated sites did not have the dominant subpopulation as their single greatest membership assignment. Among MX sites, 48.3% of individuals were identified with a single population, ≥ 3 at every site. All but two MX sites had individuals assigned to two or more subpopulations.

Grouping dominated sites by subpopulation provided a highly coherent structure. Variation among subpopulations accounted for 12.6% of variation, compared to 8.7% among sites (Table 6A). Within-site variation was slightly lower for dominated sites (78.7%) than for the general population (84.3%, Table 6C), indicating weaker genetic structure among admixed sites. The coherence of the subpopulations we identified was further validated by the scale at which spatial autocorrelation operates. Autocorrelation was extremely high between sites separated by < 15 km, and decayed to significant negative autocorrelation for

sites between 30 and 80 km apart (Figure 2). The spatial scale for autocorrelation is comparable to that of mean intersite distance within groups (Table 5).

Table 6. AMOVAs describing genetic variance partitioning among A) subpopulations, B) farms, and C-F) environmental variables

Grouping factor	Source of variation	df	Variance component	Variance (%)
A) Dominant subpopulation	Among groups	3	1.56*	12.6%
	Among sites within groups	36	1.08*	8.7%
	Within sites	927	9.73*	78.7%
B) Farm	Among groups	19	1.22*	10.4%
	Among sites within groups	22	0.65*	5.6%
	Within sites	898	9.81*	84.0%
C) Shading	Among groups	1	0.00	0.0%
	Among sites within groups	69	1.83*	15.7%
	Within sites	1578	9.79*	84.3%
D) Tree adjacency	Among groups	1	0.05*	0.4%
	Among sites within groups	69	1.79*	15.3%
E) Use as pasture	Among groups	1	0.01*	0.1%
	Among sites within groups	69	1.82*	15.7%
F) Slope	Among groups	2	0.02*	0.2%
	Among sites within groups	68	1.79*	15.4%

Variance within sites is identical for factors C-F so is only represented once. A) contains only individuals located in dominated sites, B) contains only individuals from farms with multiple sample sites, C-F) contain all individuals in the study.

* indicates significance ($p = 0.001$, 999 permutations).

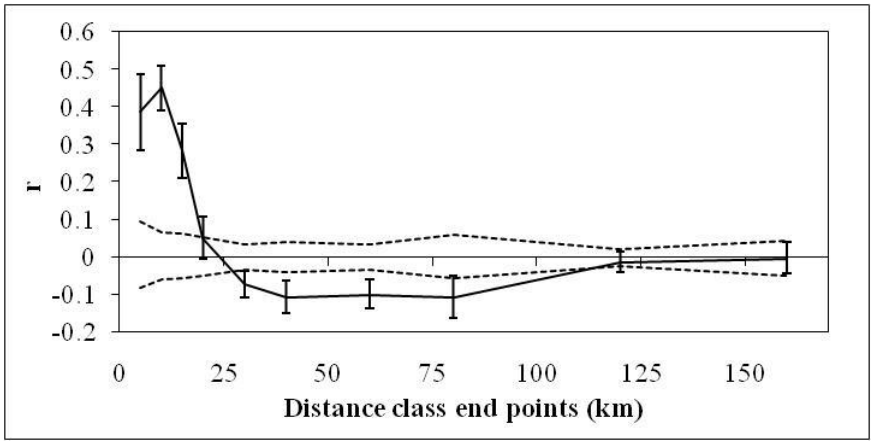

Figure 2. Spatial autocorrelation between sites.

Dotted lines indicate the 95% limits of the confidence interval for the null hypothesis of no spatial structure, determined by 9999 permutations across the dataset. Error bars are the 95% confidence interval for correlation (r), determined by 10000 bootstraps.

Haplotype presence and structure

All samples were screened for the characteristic MFhapC marker, which was absent in all cases, suggesting the C haplotype is either missing or extremely rare in this region. Concurrently, samples were screened for the novel MFhapD marker (Table 4 and Appendix 3). We identified 58 individuals at 4 sites (WC-POB-LF, WV-PIG-LH, MH-267-LF, MH-267-LH). Additionally, we assayed individuals from 34 sites for the presence of the MFhapA marker; 43.4% scored positive for the marker. The full spectrum of admixture is represented within these sites, from fixation of the A haplotype to its complete absence (Appendix 3). Thsu, we were able to verify the presence of all known haplotypes except haplotype C.

At the site level, we detected a strong correspondence between haplotype and subpopulation identity. IA-dominated sites clearly had much higher proportions of the A haplotype than admixed sites or those dominated by other subpopulations (Figure 3, no

LX-dominated sites were tested). IA-dominated sites had a mean haplotype A proportion of 0.817 (SE +/- 0.054) compared to 0.031 (SD +/- 0.024) for WI and GR sites combined and 0.265 (SE +/- 0.092) for MX sites. A chi-square test of independence confirmed these groups had significantly different haplotype A proportions ($p < 0.001$). Likewise, the sites containing the D haplotype were the same four dominated by the LX subpopulation, linking that subpopulation and haplotype (Figure 1). Neither linkage is present at the individual level within sites, however. LX-dominated sites had at least two haplotypes present; individual plants with the D haplotype were not more strongly assigned to the LX population at any site, and in sites with haplotype admixtures plants with the A haplotype are not more strongly assigned to the IA population (chi-square tests of independence, all $p > 0.05$),

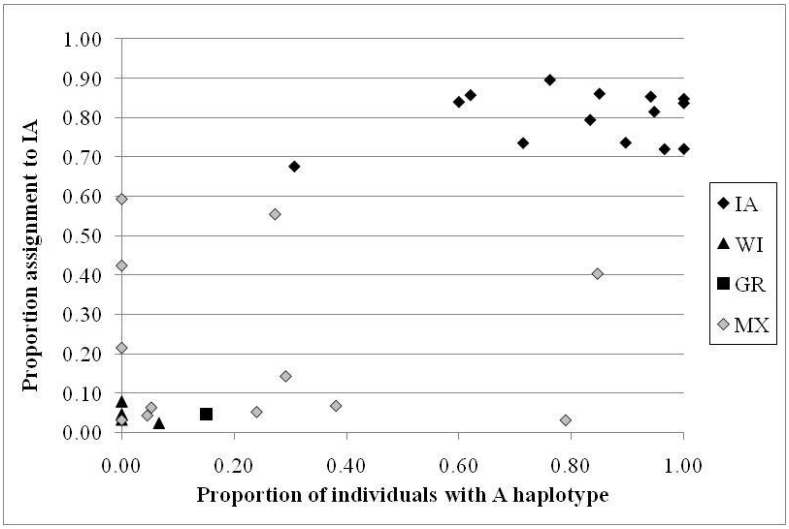

Figure 3. Correspondence between IA subpopulation and haplotype A across 34 sites.

Genetic diversity patterns

The 20 loci used in this study had a total of 191 alleles. Of these, 84 (44.0%) had overall frequencies < 0.01, considered rare following Peter-Schmid *et al*. (2008a). The number of rare alleles per plant varied considerably between sites, with a global mean value

0.30 (SD 0.17), while the mean number of alleles per locus was slightly more consistent, with a mean across sites of 4.04 (SD 0.62). Mean values of unbiased expected heterozygosity (UHe) across loci, a more general measure of overall genetic diversity, showed less variation, with a mean of 0.469 (SD 0.042).

Genetic variation differed among dominance categories, with separate ANOVAs detecting significant differences in alleles per locus, UHe, and polymorphism among the categories (all $p < 0.001$). Sites dominated by WI had the lowest diversity, with fewer alleles per locus and lower UHe than any other group, while IA dominated sites tended to have more diverse, with significantly higher polymorphism than every other site grouping but LX (Table 5). WI-dominated sites also had the lowest rate of rare alleles, although differences among sites were not significant ($p = 0.100$). As a subpopulation, WI also had a much higher level of fixation ($F_{st} = 0.123$) than the other three subpopulations (mean $F_{st} = 0.061$, SD 0.008).

Environmental effects and within-site spatial structure

Sites were grouped by four environmental parameters: shading, slope, use as pasture, and proximity to trees (Table 1). Although separate AMOVAs confirmed all groupings except shade had statistically significant explanatory power, the variance explained by each was miniscule compared to differences among sites within each category (Table 6 C-F).

Spatial autocorrelation of plants at individual sites found no significant or interpretable structure at 42 sites and identified 29 sites with one of 2 structural patterns, although these were not necessarily significant at individual sites (data not shown). Combined spatial autocorrelation detected two subtly different spatial structures at a combined 29 sites, although individually most of these lacked a significant structure (Figure

4). The first structure was present in 14 sites and had marginally significant correlation for plants within 0.8 m, decaying to significant negative correlation for plants from 0.8 to 1.2 m (Figure 4A). The second structure again had a significant correlation within 0.8 m, but had a negative correlation at 1.2 to 1.8 m (Figure 4B). For both structures, even statistically significant r values were extremely low ($r < 0.1$).

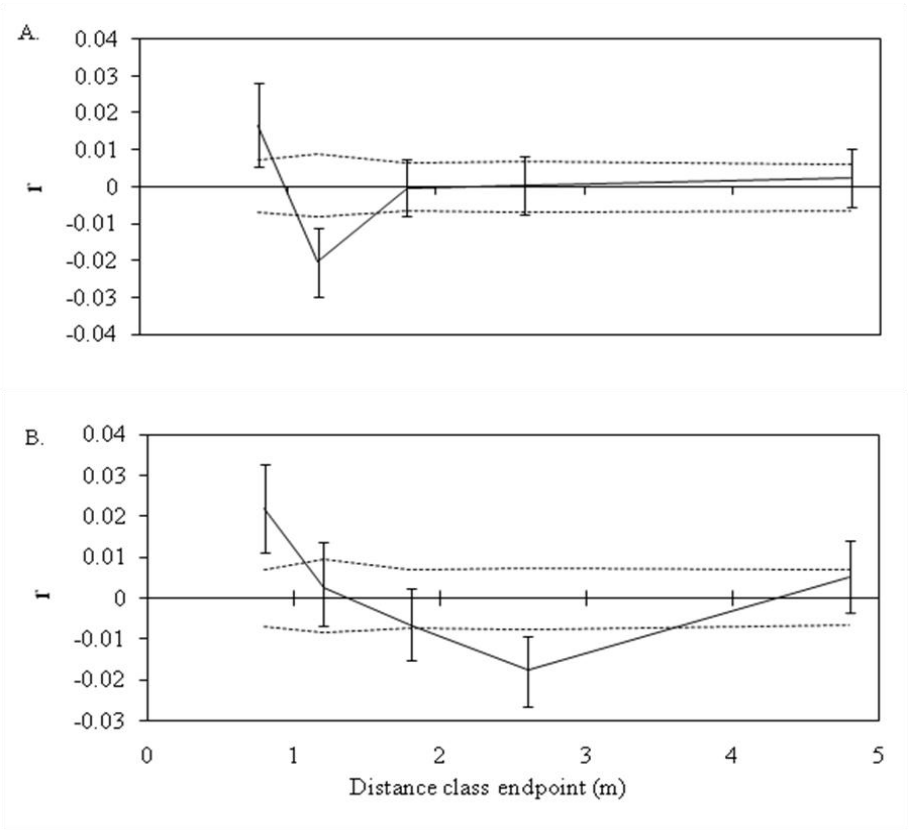

Figure 4. Patterns of within-site spatial autocorrelation.

Patterns implied A) short range dispersal (n = 14), and B) medium range dispersal (n = 15). Dotted lines indicate the 95% limits of the confidence interval for the null hypothesis of no spatial structure, determined by 9999 permutations. Error bars are the 95% confidence interval for correlation (r), determined by 10000 bootstraps.

Discussion

Substantial diversity present in Driftless Area

The levels of genetic diversity and its partition within and among populations is roughly comparable to values reported for Swiss and Norwegian meadow fescue populations (Fjellheim, Rognli, 2005; Peter-Schmid *et al.*, 2008a). While different sampling approaches and marker systems preclude direct comparison of results, some general conclusions can be drawn. Although low by many species' standards, levels of expected heterozygosity in Driftless Area meadow fescue was very similar to what was found in Swiss populations by Peter-Schmid *et al*. (2008a). Perhaps more tellingly, for all but one of the loci common to both studies, the number of alleles we identified was equal to or greater than the number reported within the Swiss populations. We take this as evidence that the range of genetic diversity present in Driftless Area meadow fescue is on the same scale as that found in a similarly sized area in the heart of meadow fescue's native range (Table 2). Populations typically lose much of their genetic diversity soon after being separated from core populations, due to a mixture of small population sizes and isolation (Jacquemyn *et al.*, 2009). Barring substantial, unreported propagule flow from Europe, the retention of diversity in Driftless Area meadow fescue suggests niches capable of supporting large meadow fescue populations (Silvertown *et al.*, 2009). The abundance of rare alleles we identified can be explained either by extremely effective sampling of the diversity present in the original populations, or by the creation of novel alleles since the arrival of meadow fescue in this region.

Subpopulation structure suggests different introduction and fragmentation histories

We found evidence for four genetically distinct subpopulations, three with discreet geographic distributions (Figure 1). For all subpopulations we identified multiple sites that were dominated (proportional membership > 0.75) or admixed (two or more proportional memberships > 0.20). Grouping individuals by subpopulation explained most of the genetic variability among sites, although sites dominated by the same subpopulation retained some variation amongst themselves (Table 6A). This contrasts with observations in Norwegian meadow fescue populations, where variance within groups was much higher than variance among them (Fjellheim, Rognli, 2005).

The genetic basis for the subpopulations was at least partially determined by allelic composition. There were 73 alleles (38.2%) that were entirely absent from at least one subpopulation, indicating the subpopulations were not simply defined by different abundances of a common set of alleles (compare to Van Treuren *et al.*, 2005). Similarly, we expect qualitative differences among the subpopulations in adaptive genetic diversity.

Among the subpopulations, WI is an outlier by many metrics. As a subpopulation, its F_{st} estimate is nearly double that of the other subpopulations, indicating a much greater degree of differentiation among sites. Likewise, WI-dominated sites are significantly less diverse than those dominated by other subpopulations or admixed (Table 3). These sites also lack the clear geographic distribution observed for other subpopulations (Figure 1). The location of most of these sites at the edges of the study area raises the possibility that those sites are a recently colonized periphery, which typically has greater differentiation and lower diversity (Eckert *et al.*, 2008). Peripheral sites, however, are expected to be genetically similar to the core population, but not to each other (Parisod, Bonvin, 2008), while the WI sites maintain a similar genetic identity despite their greater mutual separation (Table 5). We

speculate instead that the WI subpopulation the remnant of a single population that covered the study area prior to the introduction of the other subpopulatoins. Sites with a longer history of colonization frequently have greater differentiation among themselves than those more recently settled (Jacquemyn *et al.*, 2004). The reduced within-site diversity of WI dominated sites is likely to be the result of historical contraction and fragmentation, similar to dynamics posited for *Geum triflorum* by Hamilton and Eckert (2007).

At the site level, subpopulations coincide with specific haplotypes (Figure 3). The different meadow fescue haplotypes are thought to have originated from separate refugia during the last glacial-period and have largely distinct geographic ranges (Fjellheim *et al.*, 2006). The presence of multiple haplotypes in the Driftless Area demonstrates different European sources are present, while their connection to distinct subpopulations demonstrates a largely intact genetic identity and argues for separate introductions. As distinct haplotypes correspond to different gene pools in Europe (Fjellheim *et al.*, 2009), the groups present in the Driftless Area sample distinct, historically separated sources of meadow fescue genetic material. This confluence of genetic material from throughout the species range should prove to be an interesting natural breeding experiment and may result in novel and useful combinations.

Decreased allelic richness and polymorphism have both been shown to relate linearly to the log of effective population size Allele (Young *et al.*, 1996), so these parameters can serve to compare size-related constraints on genetic diversity between groups. By this metric, IA and LX-dominated sites draw upon similarly sized populations, even though the latter are substantially fewer and farther apart (Table 3). It is unlikely that meadow fescue is much more abundant, and meadow fescue pastures more connected, in the northern part of our

study range. Thus, it would appear the LX subpopulation represents a large, diverse introduction that occurred relatively recently, in species-relative terms. Obligate outbreeding species like meadow fescue tend to maintain high levels of heterozygosity (Cornish *et al.*, 1979). There is some evidence that meadow fescue plants in the Driftless Area may survive 10 years or more, which is exceptional longevity for a pasture species (Casler *et al.*, 2008). Together, these traits suggest a mechanism by which the rate of genetic drift in meadow fescue would be closer to that of a tree than an annual grass (Austerlitz *et al.*, 2000). If this is true, the predicted rate at which genetic diversity arises would be relatively low, suggesting most of the range of diversity we observe was present in the founding population.

Limited gene flow between sites

We found weak evidence that dispersal, particularly by seed is limited among sites. Across organisms, admixture between differentiated populations in novel habitats results in greater diversity than is present in the original sites (Petit *et al.*, 2003; Simon-Bouhet *et al.*, 2006), leading us to predict admixed sites would have higher diversity than dominated sites. This was, however, not the case, with MX sites not significantly different from every group but WI, and with significantly lower allelic richness and polymorphism rates than sites dominated by the IA subpopulation (Table 5). A combination of spatial and temporal isolation can explain this result. The decoupling of haplotype frequency from IA assignment in MX sites (Figure 3) could signify a limited contribution of seeds to the admixture of sites, and limited seed dispersal overall. At the same time, we observed relatively high numbers of individuals with clear, and distinct, subpopulation assignments within admixed sites. Population assignment is a continuous, rather than discreet, characteristic that is irreversibly eroded by outbreeding, so preservation of distinct population identities would require low

generational turnover relative to genetic inflow from strongly dominated sites. As previously discussed, longevity of individual plants could effectively reduce the turnover rate. The spatial structure we found within sites (Figure 4) was much weaker and occurred at a much smaller scale than was described for the self-incompatible herb *Biscutella laevigata* by Parisod and Bonvin (2008). This observation is also consistent with a long individual lifespan, as the offspring of a single plant would make up a very small proportion of its neighbors.

Effects of historic land use changes are visible in current population structure

Historical landscape changes have an effect on current population structure (Jacquemyn *et al.*, 2004). Some of our observations, particularly in the WI subpopulation, may be rooted in landscape-level changes in agriculture that have occurred in the Driftless Area over the last 180 years.

Intensive European settlement of the Driftless Area began in the 1830's; the replacement of existing prairies and oak savannas with pastures and fields created habitat suitable for meadow fescue colonization (Knox, 2001). While meadow fescue may have been present since that time, it was more likely introduced in the 1890's, when it was used extensively throughout what is now the tall fescue belt, and could have been transported to Wisconsin with cattle from that region (Hein, 1947). By the 1910's, however, meadow fescue seed production and trade were virtually nonexistent, and found almost exclusively in parts of Kansas and Missouri (Holmes, 1913). Potential meadow fescue habitat in the Driftless Area contracted sharply beginning in beginning in the 1930s, when row crop acreage in the region increased substantially, largely at the expense of grasslands (Helms *et al.*, 1996).

The ability of meadow fescue to persist despite the loss of habitat and lack of human-mediated propagation may have partially been due to the irregular topography and less intensive agriculture in the Driftless Area, which created fragmented pockets that went untilled and unseeded (Knutson *et al.*, 2004). It is likely many of these pockets were remnant oak savannas, given the frequency with which they are found near meadow fescue patches (Casler *et al.*, 2008). Soil conservation practices begun in the 1930's included fencing cattle away from wooded hillslopes, which would have further isolated these patches by reducing the potential role of animal vectors for transportation (Juckem *et al.*, 2008). The small patch size and hindrances to gene flow likely led to reduction in genetic variability and differentiation among these remnant sites.

Of the subpopulations we identified, WI shows the greatest signs of having undergone population fragmentation and reduction, as evidenced both by its increased differentiation among sites and reduced diversity within sites (Table 5). The absence of these traits among other subpopulations could indicate better connectivity among sites or a more recent introduction, allowing less time for divergence. While we lack conclusive support for either possibility, the presence of WI-dominated sites across the outer edges of the study area is suggestive of separate introduction phases (Figure 1). The current WI-dominated sites would represent the extent of expansion in the first phase while diffusion from sites of introduction in the second phase is currently ongoing.

If the WI subpopulation truly was restricted in its habitat to remnant oak savanna, current its current gene pool the result of a 50-year natural selection experiment. Meadow fescue is thought to respond poorly to low light conditions, particularly under intensive management, (Zimmermann, Nosberger, 1999) so plants that survived these conditions are

likely to have adapted accordingly. We attempted to test this hypothesis by sampling both from shaded sites and those in full sunlight within the same farm. We found no differences in genetic diversity between the two site types, and grouping sites by their shading status did not explain any genetic variability. These results are inconclusive, however, as we had no means by which to determine whether the sun sites had been colonized from the shade or vice versa. We found environmental features did not explain variance in a meaningful way suggesting there is limited selection for microsite conditions (Table 6). Regardless of the nature of the refugia, our results suggest the WI subpopulation has had considerable exposure to local selective pressures, making these plants likely sources of useful local adaptations.

Conclusions

The Driftless Area shows signs of being an excellent source of meadow fescue genetic diversity. Maintenance of diversity in productive on-farm systems provides a useful *in situ* reservoir of diversity, which can continue to adapt to changing conditions (Love, Spaner, 2007; Maxted *et al.*, 2002). Moreover, as environmental conditions continue to change in unpredictable ways, the option value of genetic diversity becomes increasingly important (Jump *et al.*, 2009; Wilkins, 1991). Meadow fescue populations in the Driftless Area are sufficiently large to maintain this diversity over time. The meadow fescue populations here have established in a novel and dynamic landscape, so these populations should be well suited to handle environmental change.

At the same time, the potential for localized adaptation may be ephemeral and easily disrupted if actions are taken to facilitate propagule movement between sites. Localized natural selection can lead to useful adaptive diversity, and even among existing meadow fescue cultivars there is evidence of significant genotype by location interactions for seed

production (Fang *et al.*, 2004; Loos, 1994). However, increased gene flow can counteract or reverse local adaptation (Lenormand, 2002). High connectivity between ostensible reservoirs of diversity and sites managed for productivity rather than diversity can lead to homogenization of allelic diversity across the landscape (Van Treuren *et al.*, 2005). Additionally, there is a risk of outbreeding depression of poorly adapted plants are introduced into a locally adapted population (Edmands, 1999; Montalvo, Ellstrand, 2001). Thus, while the Driftless Area meadow fescue is a valuable resource for maintaining and increasing diversity in meadow fescue, care must be taken to ensure that in tapping that resource we do not erode its advantages.

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Chapter 2.

Effect of nitrogen and cutting height on competitive interactions between meadow fescue and cool-season pasture grasses is species specific

Introduction

Meadow fescue (*Festuca pratensis* Huds.) is a perennial bunchgrass whose potential for high quality cool season pastures is generally unrealized in the North Central USA. It consistently has a higher forage quality than other common grass species in this region, which translates into improved animal performance (Brink *et al.*, 2008). This quality, combined with its winter hardiness and drought tolerance, make it an important forage species in cooler pastures throughout the world (Carlen *et al.*, 1999). Meadow fescue has already naturalized to the southern parts of the Driftless Area (Casler *et al.*, 2008), and can be a substantial component of the species assemblage in localized patches (Appendix 4). This grass seems particularly well adapted to the rotational grazing management style that is being increasingly adopted in this region (Casler, van Santen, 2001).

Although optimal management conditions for a homogenous stand are being investigated (Brink and Casler, unpublished data), meadow fescue is more likely to be incorporated into production systems as part of a mixture of species. Preservation of biodiversity is increasingly an important element in grassland management, both as a means to achieve the multiple demands being placed on grass-based agroecosystems and as an end in itself (Sanderson *et al.*, 2004). Meadow fescue has shown itself to be a poor competitor in European semi-natural grasslands, faring poorly in direct competition with common pasture grasses (Carlen et al., 1999; Carlen et al., 2002) and experiencing drastic reductions in

biomass when crowded (Zimmermann, Nosberger, 1999). Given these potential deficiencies, it would be useful to determine whether common management styles can be adapted to improve meadow fescue persistence in cool season pastures.

Considerable attention has been devoted to adapting management to encourage persistence of legumes, emphasizing defoliation and nitrogen management (Barthram *et al.*, 1992; Mitchell, Nelson, 2003). The timing and frequency of cutting have been shown to affect species composition in diverse grasslands (Kramberger, Kaligaric, 2008) while increased nitrogen availability strongly favors certain species at the expense of others (Tilman, 1987), commonly benefiting exotic grasses over native species (Kolb et al., 2002). We chose to test how the interaction of defoliation height and nitrogen addition affects meadow fescue's ability to compete with other cool season perennial grasses. To avoid confounding from meadow fescue's established management-dependent response to water and shading stress (Zimmermann, Nosberger, 1999), we designed this as a greenhouse experiment where we could control moisture and lighting conditions.

From a survey of sites containing meadow fescue in the Driftless Area, we determined the grass species most commonly found growing alongside meadow fescue were Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*F. arundinacea* Schreb.), quackgrass (*Elymus repens* L.), and timothy (*Phleum pretense* L.), while red clover (*Trifolium repens* L.) was a commonly coinciding legume (Appendix 4). Given the interest in meadow fescue persistence within diverse communities, we tested meadow fescue growth in a mixture of the grasses as well as a mixture of the grasses and clover, in addition to testing meadow fescue in pairings with each of the grasses. The grass species in this study represent a range of persistence in mixtures; timothy is moderately effective competitor, Kentucky bluegrass and

tall fescue both excellent and quackgrass an extremely aggressive weed (Balasko, Nelson, 2003). Timothy persistence is negatively impacted by close grazing, particularly with high nitrogen levels (Berg et al., 1996). In contrast, Kentucky bluegrass is known for its ability to persist under close grazing, but establishes slowly and is an indifferent competitor for nitrogen (Wedin, Huff, 1996). Both tall fescue and quackgrass have a limited tolerance for close grazing and respond positively to nitrogen (Balasko, Nelson, 2003).

Because we were primarily interested in determining how management affects the outcome of meadow fescue's competitive interactions with other species, we employed a relative competition index (RCI) based on the ratio of meadow fescue production in mixtures and monocultures receiving the same management. By relativizing production within management, the RCI eliminates the effect of productivity differences between management styles, allowing for a comparison of interspecific and intraspecific competition that is independent of the level of production (Grace, 1995).

Our goals for this study were to determine whether the interaction of defolation height, nitrogen addition, and competitor species can result in significant differences between interspecific and intraspecific competition for meadow fescue, and whether differences in competitive outcomes arise from improved coexistence or displacement.

Materials and Methods

Plant Materials and Growing conditions

Species used in this study were Kentucky bluegrass (cv Ginger), tall fescue (cv Barolex), timothy (cv Summit), red clover (cv Start), meadow fescue (open pollinated, collected from a farm in Iowa County, WI), and quackgrass (WRFQ, described in Casler and Goodwin (1998)). Plants were grown in standard 30-cm pots filled to approximately 25 cm

with a 1:1 mixture of composted field soil (pH 7, OM 5.8%, P 165 ppm, K 500 ppm) and medium sand, with a final texture of approximately 75% sand and 11% clay by weight. Plants were grown in a greenhouse with a 16-hour supplemental photoperiod from 400-W high-pressure sodium lamps and an average temperature range of 18.3 to 26.7 ºC.

Treatments

Meadow fescue was grown in one of seven competition treatments: as a monoculture (MO), in pairwise competition with tall fescue (TF), Kentucky bluegrass (KY), quackgrass (QK), or timothy (TM), or with a mixture of all grasses (GM) or all grasses and red clover (CM). In all treatments, a total of 650 live seed per m^2 were planted in each pot. In all treatments but MO, meadow fescue comprised 1/6 of the live seed in each pot, with the remainder divided evenly among the competitor species.

Two levels of defoliation were studied: removal of biomass to either 5- (D05) or 10-cm (D10) above the surface. The defoliation treatment was first applied 7 weeks after planting, then every 4 weeks after. The experiment concluded after the 4^{th} regrowth cycle, when a majority of pots were determined to be root bound. A no defoliation control (D00) was also included, but in general was analyzed separately.

Two levels of nitrogen were considered: no nitrogen amendment (N00) or 60 kg/ha supplemental nitrogen spread over two applications (N60). Nitrogen was supplied as aqueous ammonium nitrate at 7 and 15 weeks after planting, coinciding with the 1^{st} and 3^{rd} defoliation treatments.

All treatment combinations were included, with 28 treatments (7 compositions x 2 defoliation intensities x 2 nitrogen levels) in the primary experiment and 14 treatments (7 compositions x 2 nitrogen levels) in the no defoliation (D00) controls. All treatments were

replicated 5 times, with replicates placed on different greenhouse benches in a randomized complete block design. One replicate of the KY-D05-N60 had no meadow fescue germination and was removed from the experiment.

Measurements

Emerged tillers were counted 3 weeks after planting as an indication of germination success for inclusion as a covariate in subsequent models. Immediately before the final defoliation treatment, relative species abundance was determined for each pot by a modified point-intercept method. For each of 25 points evenly spaced on a grid 25 cm on a side, the first canopy hit was identified to the species level or marked as bare if no plant covered that point.

At the conclusion of the 4th regrowth cycle, all aboveground biomass was collected from the pots. Biomass was separated into meadow fescue and non-meadow fescue portions. For pots receiving D05 and D10 treatments, biomass was further divided into regrowth (biomass at and above the defoliation height) and residue portions. After these subdivisions biomass was handled separately. Biomass was dried at 65 ºC for a minimum of 48 hours before weighing.

Metrics and statistical analysis

Biomass values for MO pots were transformed by a factor of 0.1667 when comparing meadow fescue production among competition treatments to correct for differences in meadow fescue seeding rate. Relative competition intensity (RCI) was modified from Turkington et al. (1993). RCI is calculated at the experimental unit level as

$$\mathrm{RCI}_{i,j,k} = \ln\left(\frac{MI_{i,j,k}}{MO_j}\right)$$

where $MI_{i,j,k}$ is meadow fescue biomass produced by the *i*th replicate of the *j*th management style applied to the *k*th composition and MO_j is the arithmetic mean of biomass (transformed) produced by the meadow fescue monoculture under the *j*th management style. Positive values indicate a release from competitive pressures relative to monoculture, while negative values correspond to increased competition intensity. Significance of departure from 0 was tested by a heteroscedastic two-sample *t* test between a mixture and the monoculture within a management style. Log transformation of the yield ratio was done to give equal weight to reductions in yield, making this metric equally capable of detecting increases and decreases in competitive intensity.

Production of meadow fescue and non-meadow fescue biomass, as well as relative abundance of grass species in the two mixture compositions, was analyzed to characterize the growth and competition dynamics underlying the changes in competition intensity. Analyses were conducted with Proc MIXED in the SAS program (version 9.1.3 service pack 3). Models incorporating defoliation, nitrogen, and competitor species composition, and all of their interactions, as well as greenhouse bench as a random blocking factor and initial tiller count as a covariate were run separately for meadow fescue and non-meadow fescue aboveground biomass from D05 and D10 treatments to determine the highest level of significant interaction. Subsequently, separate ANOVAs were run for each composition testing the effect of nitrogen, defoliation, and their interaction meadow fescue and non-meadow fescue biomass. Significant differences for management styles within compositions were determined by significance testing of differences in least squares means. Fully factorial ANOVAs were run separately for regrowth and residue portions of meadow fescue biomass to verify that their responses to treatments were qualitatively equivalent. Meadow fescue and

non-meadow fescue biomass from the D00 treatment were analyzed separately, modeling the effects of nitrogen and competitor species composition. Finally, relative abundance in the GM and CM compositions was modeled for each grass across all defoliation and nitrogen managements to estimate the response of the individual species to management effects.

Results

Changes to competition intensity

Several combinations of management and competitor composition resulted in positive RCI values, indicative of significant competitive release for meadow fescue (Figure 1). With the Kentucky bluegrass (KY) composition, this release was significant at the $p < 0.05$ level for all management combinations except with 10-cm residual stubble and no added nitrogen (D10-N00, $p = 0.18$). Inversely, the tall fescue (TF) and quackgrass (QK) compositions each had only one management combination that significantly lightened the competitive pressure experienced by meadow fescue: D05-N00 for the TF composition and D10-N00 for the QK composition ($p = 0.02$ and $p = 0.01$). In addition, the D10-N60 management with the grass mixture (GM) composition and the D05-N60 management with TF were marginally significant ($p = 0.08$ and $p = 0.07$). There were no significant changes in competition intensity for the timothy (TY) and clover mixture (CM) compositions and no treatment increased competition intensity relative to the corresponding monoculture (Figure 1). Large, but insignificant, negative values were observed for QK-D10-N60, TY-D05-N60, and GM-D10-N00. These values were driven by single, extreme measurements, revealing a tendency in this metric to emphasize low production values.

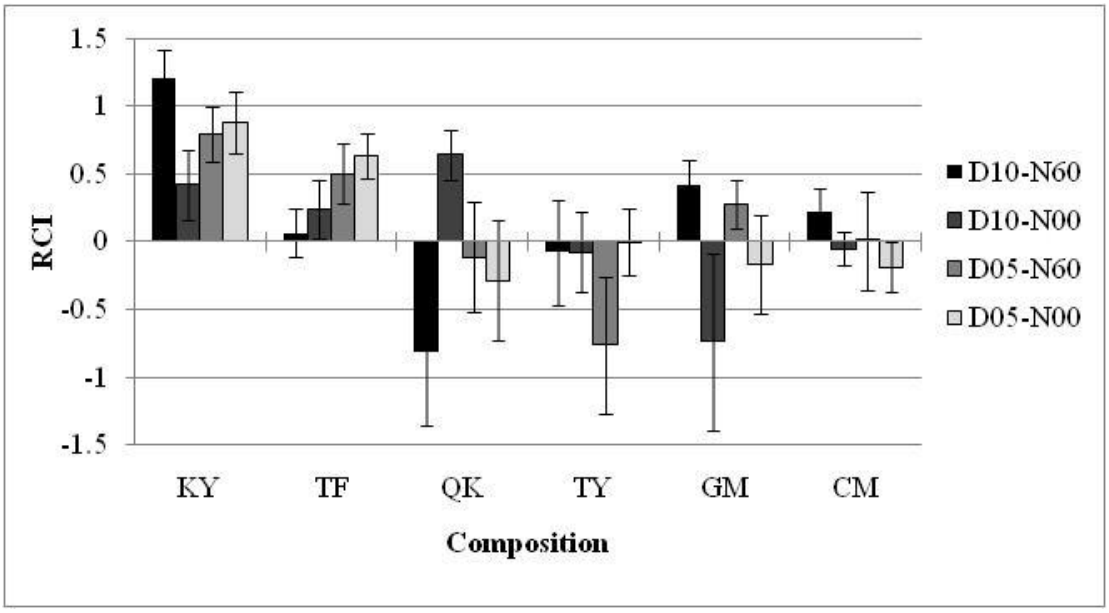

Figure 1. Relative competitive index (RCI) values for for Kentucky bluegrass (KY), quackgrass (QK), tall fescue (TF), timothy (TY), a grass mixture (GM) and a grass-clover mixture (CM), with residual stubble height of 10 cm (D10) and 5 cm (D05) and with (N60) and without (N00) added. Error bars are ± 1 SE. Values greater than 0 indicate a release in competitive pressure relative to a meadow fescue monoculture.

Growth and competition dynamics

Aboveground standing biomass in the pots receiving the defoliation treatments served as an integrative metric of ability to regrow after repeated defoliation. The interaction of nitrogen, defolation height, and competitor species identity significantly impacted meadow fescue biomass ($p < 0.01$), as did the main effects of defoliation height, competitor species, and the interaction of competitor species and nitrogen (all $p < 0.05$). By contrast, non-meadow fescue biomass was impacted by the interaction of defoliation height and composition ($p = 0.04$), as well by their main effects (both $p < 0.01$), but no effect of nitrogen was observed.

To facilitate interpretation of the higher order interactions we observed, we conducted

analyses of the effect of nitrogen and defoliation on both meadow fescue and non-meadow fescue biomass separately for each composition treatment (Table 1). There was a significant effect of nitrogen and defoliation on meadow fescue biomass in the QK composition (p = 0.03), as well as a marginally significant effect on meadow fescue in the KY treatment (p = 0.08) and on competitor biomass in the GM treatment (p = 0.07). In the QK composition, the D10-N00 management had significantly greater meadow fescue biomass than each of the other managements (Figure 2A, $p < 0.05$). For the KY composition, the D10-N60 management had significantly more meadow fescue biomass than the others ($p < 0.05$). There was no significant interaction between nitrogen and defoliation in the TF composition, making it the only treatment with a significant impact on RCI that did not impact meadow fescue biomass. Among competitor compositions, only GM displayed a significant nitrogen x defoliation effect, with the D10-N00 management producing more non-meadow fescue biomass than any other management (Figure 2B, $p < 0.05$).

Table 1. *P* values from two-way ANOVAs modeling the effects of nitrogen (N) and defoliation (D) on meadow fescue and non-meadow fescue biomass

Component	Factor	KY	QK	TF	TY	GM	CM	MO
Meadow fescue	N	0.08	0.11	0.65	0.98	0.15	**0.05**	0.52
	D	**0.02**	**0.02**	**0.02**	**0.03**	**0.02**	**<0.01**	**<0.01**
	N x D	0.08	**0.03**	0.62	0.98	0.16	0.58	0.65
Non-meadow fescue	N	0.10	0.52	0.42	0.43	0.19	0.14	
	D	0.15	**0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	
	N x D	0.24	0.68	0.66	0.34	0.07	0.25	

Significance for the covariate of initial tillering are not included. Bolded numbers are significant with $p < 0.05$.

The main effect of defoliation was consistent across nearly all compositions, leading to increases in biomass both for meadow fescue and competitor species that were significant in every case except that of Kentucky bluegrass in the KY composition (p = 0.15, Table 1).

Nitrogen had a marginally significant positive effect on meadow fescue growth in the CM (p = 0.05) and the KY (p = 0.08) mixtures (Figure 2A).

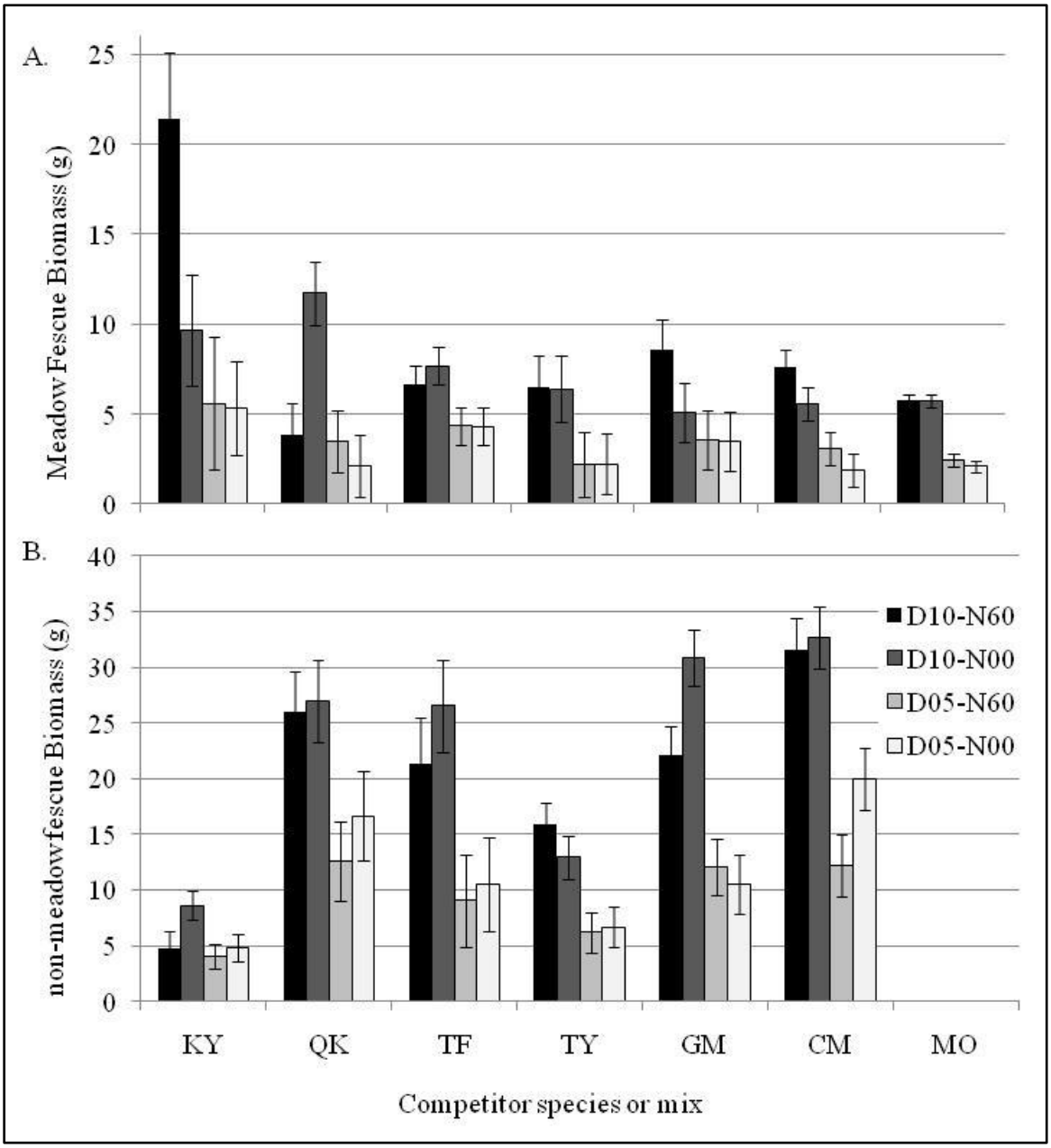

Figure 2. Aboveground biomass of meadow fescue (A) and competitor species (B), with and without supplemental nitrogen (N00, N60) and with 5- or 10-cm residual (D05, D10) grown in competition with Kentucky bluegrass (KY), quackgrass (QK), tall fescue (TF), timothy

(TY), a grass mixture (GM), a clover-grass mixture (CM), or as a monoculture (MO). Treatment means and errors were calculated separately for each competitor species or mix. Error bars represent 1 SE, n = 5.

Dynamics of competition

The residual and regrowth portions of the meadow fescue biomass responded very similarly to the different managements and competitions (Table 2). A notable exception occurred with the meadow fescue under D10-N00 management with the QK composition, which was significantly more productive than the D10-N00 monoculture in the residual ($p < 0.01$) but not in the regrowth ($p = 0.13$), suggesting a substantially lower growth habit.

Table 2. *P* values from two-way ANOVAs modeling the effects of nitrogen, defoliation, and composition on the regrown and residual components of meadow fescue biomass

	Regrowth		Residue	
Factor	F Value	P value	F Value	P value
N	3.6	0.06	0.6	0.45
D	**47.1**	<0.01	**75.3**	<0.01
C	**7.5**	<0.01	**7.8**	<0.01
N x D	0.5	0.49	0	0.99
N x C	**2.7**	0.02	**2.8**	0.02
D x C	1.7	0.13	1.6	0.16
N x D x C	**3.1**	<0.01	**3.7**	<0.01

Residue height is determined by the defoliation treatment, with D05 and D10 corresponding to 5- and 10-cm, respectively. Values in bold are significant at $p < 0.05$.

Competition in the absence of defoliation

In contrast to the defoliation treatments, which measured capacity for regrowth, the biomass in undefoliated controls (D00) served as an indicator of the species' ability to establish and compete for resources with similarly established individuals. In the D00 management, meadow fescue biomass was significantly affected by composition ($p < 0.01$),

but not nitrogen ($p = 0.34$) or the interaction of nitrogen and composition ($p = 0.48$). In the absence of defoliation, production of meadow fescue biomass was strongly and inversely linked to competitor biomass (Figure 3). The KY and TY compositions had significantly more meadow fescue biomass than any other composition, with KY significantly greater in turn than TY (all $p < 0.05$). Conversely, QK produced less meadow fescue than any mixture except GM ($p = 0.06$), although not significantly less than the monoculture ($p = 0.21$).

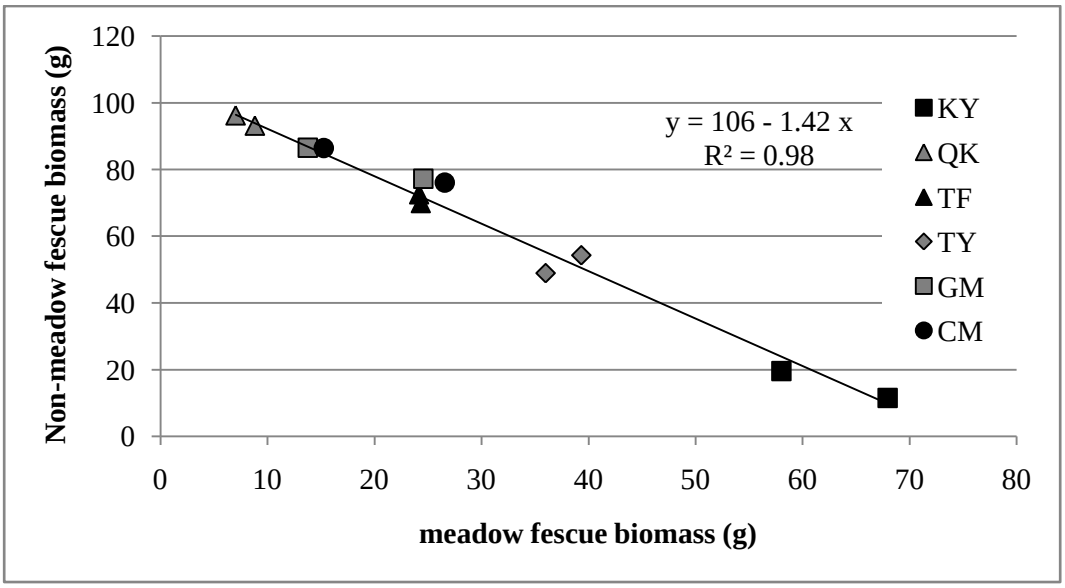

Figure 3. Regression of non-meadow fescue on meadow fescue biomass in undefoliated pots across 12 competitor and nitrogen treatments. Each point represents a mean over 5 replicates, with plotting character indicating competitor composition.

Performance of individual species in a mixture

Relative abundance of the grass species in the two mixtures (GM and CM) was modeled as a function of nitrogen, defoliation, and mixture to ascertain whether the species-specific response to management in paired mixtures would be replicated in mixtures with more components. For this analysis, all defoliation (D00, D05, D10) and nitrogen (N00, N60) treatments but only the GM and CM compositions were considered. Only tall fescue and

Kentucky bluegrass responded significantly to differences in management, both to defoliation (Table 3). Tall fescue was significantly more abundant in mixtures with D00 management than with either D10 ($p = 0.04$) or D05 ($p < 0.01$). In contrast, Kentucky bluegrass was significantly less abundant with D00 management than D05 ($p < 0.01$) but not D10 ($p = 0.09$). Though defoliation was not significant as a main effect for timothy ($p = 0.10$), its abundance was significantly higher in the D00 management than D05 ($p = 0.04$).

Table 3. ANOVAs of factors affecting relative abundance of grasses in two mixtures (GM and CM)

Factor	meadow fescue	tall fescue	quackgrass	timothy	Kentucky bluegrass
N	0.2326	0.4444	0.2557	0.7637	0.4415
D	0.6364	**0.0253**	0.9137	0.103	**0.0143**
C	0.3114	0.1026	0.9463	0.3689	0.0942
N x D	0.1909	0.2402	0.3456	0.4935	0.8453
N x C	0.5182	0.6573	0.8929	0.5888	0.8773
D x C	0.0774	0.3497	0.588	0.8396	0.0827
N x D x C	0.4581	0.522	0.7448	0.737	0.4792

Listed values are *P*-values for significance of factors. Values < 0.05 are bolded.

Discussion

Competitive outcomes differed among treatments

Our results provide evidence that management affects the outcome of interspecific competition for meadow fescue in a species-specific manner. Different management styles significantly reduced interspecific competition from Kentucky bluegrass, quackgrass, tall fescue (Figure 1), and timothy (Figure 3). RCI proved to be a useful metric for this type of analysis, detecting a significant reduction in competitive intensity for pots receiving D05 management with TF competition (Figure 1), despite the lack of significant difference in meadow fescue biomass between these pots and the monoculture under equivalent conditions (Figure 2A, $p = 0.14$). Including undefoliated controls, we found at least one set of growing

conditions for every competitor species that made them less intense competitors than meadow fescue.

Nominally, this result runs contrary to both the conventional wisdom and much of the literature. Meadow fescue is considered an inferior competitor to both tall fescue (Niemelainen et al., 2001) and timothy (Jorgensen, Nosberger, 1994), while quackgrass and Kentucky bluegrass are acknowledged as exceptionally aggressive competitors that regularly displace other species (Tesar, Shepherd, 1963). However, this study differs from previous work in that our primary emphasis was on competitive intensity, rather than directly comparing biomass or other measures of growth. While meadow fescue typically produces less biomass than other cool-season grasses (Brink *et al.*, 2008), our results indicate it may prove quite capable of persisting in a mixture, particularly with favorable management.

It is critical to note, however, that no treatment combination yielded a significant change in competitive pressure in either of the mixtures, indicating the results we observe stem from species-specific interactions (Figure 1). Increased species diversity, particularly increased evenness, has been shown to make communities less susceptible to invasion, possibly because greater diversity limits the capacity of new species to exploit environment-based advantages over individual members of the community (Sanderson et al., 2005; Tracy et al., 2004). At the same time, competition within mixtures was never significantly greater than for the monoculture (there were no significantly negative RCI values), so while the ability of management to promote meadow fescue persistence is limited, it is not placed at a significant disadvantage in a mixture.

Competition frequently results in displacement

In some cases, significant increases in meadow fescue biomass were linked to

significant decreases in the biomass of competitor species. This was particularly evident in the KY composition, where meadow fescue biomass was substantially higher than with other compositions while Kentucky bluegrass produced far less biomass (Figure 2). A similar, if less drastic, effect was observed for the GM composition with nitrogen addition at D10 defoliation, which led to a marginally significant increase in meadow fescue and significant reduction in competitor biomass ($p = 0.05$, $p = 0.03$, Figure 2). This relationship was not consistent, however, with increased meadow fescue growth in the QK-D10-N00 treatment coinciding with the highest quackgrass production, and overall low levels of timothy biomass in the TY composition failing to result in meadow fescue increases, except in the undefoliated treatment (Figure 2). It is likely that the situations with significant apparent displacement would, if continued over time, lead to competitive exclusion of the other species. While it is difficult to definitively assign causality in cases where displacement was observed, we demonstrated that management conditions that gave meadow fescue an advantage over a competitor species did not alter the success of that species, as measured by relative abundance, in a mixture (Table 3). Meadow fescue production in monoculture responded only to defoliation height, which did not provide a consistent competitive benefit (Figure 1). Together, these results indicate the dynamics we observed were derived from changes in species interactions rather than a general unilateral response by one of the species.

The apparent competitive advantage meadow fescue enjoyed over Kentucky bluegrass most likely is a function of differences in germination and establishment speed (Wedin, Huff, 1996). Prior research has established meadow fescue growth is substantially enhanced by reduced competition from neighbors (Zimmermann, Nosberger, 1999). Under the D10-N60 management, meadow fescue biomass production was comparable to that

produced by several competitors, even though the seeding rate for these was five times higher (Figure 2). Conversely, timothy in the TY composition had initially high germination and productivity (data not shown), and meadow fescue was apparently unable to expand in response to decreased timothy production. These results underscore the importance of initial establishment on the ability of meadow fescue to produce and persist.

The increased meadow fescue production observed in the D10-N00 management with the QK composition coincided with dramatically altered meadow fescue morphology: plants in three of the five pots in this treatment were much shorter, with thinner leaves and a sod-like growth form that effectively excluded quack from large segments of the pots. As discussed, the biomass distribution for these plants was such that their increased biomass relative to the monoculture under the same conditions was primarily driven by their growth below the 10-cm defolation height. Some meadow fescue cultivars have a capacity for this growth form (Casler, van Santen, 2000), but generally lower cutting heights are associated with greater tiller production (Jameson, Huss, 1959). Quackgrass is known to have allelopathic properties (Schulz et al., 1994) and we speculate that some chemical exudates may have triggered this change in growth form by meadow fescue. It is not clear why this would have occurred only at that particular cutting height in the absence of nitrogen, but nonetheless suggests an extremely interesting interaction.

All grasses performed better in the D10 management than D05, which has been generally observed with pasture grasses under similar conditions (Woodis, Jackson, 2008), but meadow fescue seems particularly well suited to 10-cm defoliation. At this height, meadow fescue tillers considerably more aggressively than tall fescue or orchardgrass (Brink and Jackson, unpublished data). The lack of competitive effects observed with 5-cm

defoliation could also be an effect of the increased disturbance intensity this caused, with a subsequent reduction in the importance of interspecific competitions in establishing limits to growth (Turkington et al., 1993).

Conclusions

Our results indicate that while it may be possible to encourage meadow fescue abundance in pastures dominated by single species, management conveying competitive advantages may be difficult to generalize and be substantially less effective for more diverse pastures. Despite this, we identified several dynamics that may prove fruitful for future research. The contrast between the results in KY and TY compositions highlighted the importance of space during germination and initial establishment, and suggests providing this space might be highly beneficial for meadow fescue productivity and persistence. In addition, the results observed with quackgrass reveal a surprising potential for phenotypic plasticity, which could greatly increase meadow fescue's ability to establish.

More generally, the issues addressed by this research lie at the heart of attempts to maintain and increase biodiversity in pastures and semi-natural grasslands. Increased biodiversity can be particularly beneficial for groups of species with lower productivity (Picasso et al., 2008), but it is common for individual species to gradually dominate communities, eliminating these benefits (Sanderson et al., 2005). Given the costs of artificial maintenance, increasing the persistence of pasture communities is a critical step in improving the sustainability and profitability of these systems (Scott et al., 2000). In order to accomplish this, further research exploring management techniques that can increase persistence of individual components of the community is warranted.

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Appendix 1.
Genetic diversity and spatial metrics by site

Site	Admixture	Plants sampled	Rare alleles per plant	Alleles per locus	Percent polymorphic loci	Expected heterozygosity	Nearest dominated site (km)				within-subpopulation distance (km)
							IA	GR	LX	WI	
IA-EBD-DF	GR	28	0.46	4.40	0.85	0.492	95.0	8.7	22.3	17.0	74.3
IA-VIL-DF	GR	28	0.25	3.95	0.80	0.483	87.6	8.7	28.0	25.7	67.5
WG-61C-LF	GR	6	0.50	3.00	0.80	0.465	34.4	6.2	106.6	6.3	44.1
WG-LIM-LF	GR	20	0.30	4.25	0.95	0.523	19.7	9.1	82.3	10.8	37.0
WG-LRH-DH	GR	28	0.25	4.45	0.85	0.509	18.0	3.3	90.5	5.9	35.1
WG-LRH-LF	GR	31	0.42	4.90	0.85	0.509	17.9	3.1	90.7	5.8	35.1
WG-REB-LF	GR	28	0.29	4.60	0.80	0.525	28.1	6.2	104.5	8.3	41.3
WG-REB-LH	GR	24	0.33	4.55	0.95	0.500	28.0	6.3	104.5	8.4	41.3
WG-SHR-DH	GR	28	0.43	4.85	0.85	0.511	15.0	3.1	92.9	3.1	36.3
WG-SHR-LH	GR	25	0.32	4.40	0.85	0.507	15.0	3.1	92.8	3.1	36.3
WI-15A-LF	IA	23	0.48	4.85	0.95	0.497	4.3	27.5	116.4	10.0	13.2
WI-15A-LG	IA*	30	0.43	4.75	0.95	0.495	3.5	27.4	115.8	11.0	12.6
WI-BDR-LF	IA*	13	0.23	4.10	1.00	0.512	3.3	20.6	99.4	17.6	10.3
WI-CAE-DF	IA	22	0.23	4.35	1.00	0.503	1.7	28.4	110.9	14.4	10.4
WI-CaE-DH	IA	28	0.14	4.50	0.95	0.511	1.6	28.5	111.0	14.4	10.4
WI-CAE-LF	IA	21	0.43	4.50	0.95	0.500	1.7	28.5	110.8	14.3	13.8
WI-CAT-DG	IA	25	0.32	4.60	1.00	0.517	3.5	26.0	98.8	19.7	11.0
WI-EBU-LF	IA	28	0.29	4.65	0.95	0.508	1.6	30.0	112.2	13.1	12.0
WI-ENL-LF	IA	31	0.32	4.50	0.85	0.468	5.0	18.0	103.4	15.3	12.7
WI-FAS-DF	IA*	14	0.36	4.45	0.90	0.543	4.1	29.7	104.0	14.0	12.7
WI-FAS-LF	IA	27	0.41	4.60	0.90	0.491	3.8	29.5	103.8	14.3	10.7
WI-GRO-LF	IA	29	0.59	4.95	1.00	0.515	3.4	22.2	102.7	19.3	10.8
WI-GRO-LH	IA*	25	0.56	5.15	0.95	0.503	3.3	22.2	102.6	19.3	10.6
WI-HEJ-DH	IA	27	0.37	4.70	1.00	0.481	5.6	22.4	108.9	16.4	11.4

WI-IG8-LF	IA*	28	0.29	4.80	0.95	0.516	5.0	15.0	98.5	12.0	17.6
WI-JOW-LF	IA	22	0.73	4.75	0.90	0.539	3.5	25.7	102.2	17.8	12.6
WI-OP1-DF	IA*	16	0.25	4.10	0.90	0.511	3.5	29.7	115.7	13.9	15.0
WL-BUO-DH	IA	29	0.28	4.55	0.90	0.504	7.0	33.9	123.4	8.9	11.9
WL-WOO-LF	IA*	27	0.19	3.60	0.85	0.466	13.4	46.7	135.3	15.1	28.4
MH-267-LF	LX	32	0.16	3.70	0.90	0.490	98.5	22.3	24.1	17.7	27.8
MH-267-LH	LX	31	0.29	4.20	0.95	0.517	98.5	22.3	24.2	17.7	27.8
WC-POB-LF	LX	26	0.65	4.85	0.95	0.531	110.4	46.4	16.4	15.7	20.3
WV-PIG-LH	LX	31	0.29	4.15	0.90	0.454	98.8	52.7	16.4	4.0	23.9
IC-LIF-LF	WI	17	0.29	3.55	0.85	0.484	85.8	59.0	97.1	32.3	84.8
MH-241-DH	WI	23	0.13	2.80	0.75	0.410	109.3	17.0	17.8	44.8	114.3
MH-241-LF	WI	30	0.13	3.10	0.80	0.421	109.2	17.0	17.7	44.6	114.2
WG-CEN-LF	WI	15	0.13	3.05	0.80	0.360	32.1	23.1	128.4	22.4	57.9
WG-ESC-LF	WI	26	0.15	3.85	0.85	0.436	12.0	3.1	93.0	23.4	51.2
WG-MUS-DF	WI	27	0.00	3.45	0.85	0.438	44.4	22.4	88.7	9.4	55.8
WG-PIK-LF	WI	16	0.13	3.50	0.80	0.435	33.9	6.3	100.4	16.6	51.5
WG-RON-LF	WI	2	1.00	1.85	0.60	0.392	53.6	28.4	90.9	9.4	60.5
WI-YLA-LF	WI	27	0.07	3.65	0.90	0.424	13.1	42.3	115.2	18.4	64.9
WL-DUH-DH	WI	22	0.14	3.55	0.80	0.404	15.6	51.3	148.2	25.5	70.4
WL-FNP-LF	WI*	27	0.07	3.05	0.85	0.434	15.2	54.6	133.5	18.4	71.2
WL-FNP-LH	WI	14	0.14	2.85	0.80	0.385	15.1	54.6	133.5	18.4	71.3
WL-TR1-DF	WI	23	0.17	3.80	0.85	0.436	8.9	27.4	121.6	23.6	57.3
WL-TR1-LH	WI	20	0.45	3.80	0.90	0.445	9.8	27.8	123.2	22.4	57.7
WV-BAG-DH	WI	27	0.19	3.55	0.80	0.447	96.9	48.9	4.0	44.8	111.7
WV-BAG-LH	WI*	26	0.35	3.90	0.90	0.485	97.1	48.8	4.0	44.6	111.8
MH-33S-DH	GR-WI	33	0.15	3.80	0.90	0.487	97.1	18.6	3.8	15.9	
MH-33S-LF	GR-WI	29	0.14	3.90	0.80	0.473	97.2	18.7	3.7	15.8	
WC-OAT-LF	GR-LX	27	0.30	4.25	0.85	0.489	112.8	54.7	8.6	15.7	

WG-BAG-LF	GR-WI	22	0.18	3.65	0.90	0.471	33.2	15.5	82.6	13.6	
WG-DRI-DF	GR-WI	24	0.42	4.40	0.90	0.511	8.6	7.1	97.2	4.8	
WG-DRI-LF	GR-WI	16	0.31	3.75	0.80	0.480	8.2	7.0	96.3	4.4	
WG-DRI-LH	GR-WI	23	0.26	4.10	0.80	0.507	8.2	7.1	96.5	4.5	
WG-ESC-LH	GR-WI	22	0.09	3.75	0.80	0.478	12.1	3.0	93.0	23.3	
WG-FWE-LH	GR-WI	27	0.04	3.80	0.80	0.461	16.9	3.0	85.2	7.9	
WG-HUV-LF	GR-WI	21	0.33	4.45	0.80	0.508	23.3	5.5	100.4	10.9	
WG-LIM-DF	GR-WI	26	0.19	3.95	0.80	0.505	19.7	9.0	82.3	10.8	
WG-RON-DF	GR-WI	9	0.22	3.60	0.90	0.484	52.7	27.7	90.7	8.4	
WG-SBK-LF	GR-WI	26	0.15	4.00	0.90	0.483	29.6	10.4	94.7	6.8	
WI-CHS-DH	IA-GR-WI	23	0.17	4.25	0.85	0.503	6.0	35.6	107.4	8.5	
WI-CHS-LF	IA-GR	23	0.30	4.50	0.85	0.516	6.3	35.6	108.2	7.9	
WI-EDW-DF	IA-GR	22	0.45	4.30	0.90	0.490	2.6	22.0	97.3	18.9	
WI-EDW-LH	IA-WI	13	0.31	3.80	0.90	0.518	2.7	22.0	97.3	18.9	
WI-OP1-LF	IA-WI	22	0.45	4.60	0.95	0.519	3.7	30.0	115.8	14.0	
WI-Y19-LH	IA-WI	6	0.50	3.10	0.85	0.480	15.1	44.1	114.6	2.6	
WI-Y19-LH	IA-WI	11	0.45	3.45	0.85	0.487	15.3	44.2	114.6	2.7	
WL-KAM-LF	LX-WI	29	0.07	3.50	0.90	0.446	6.2	43.6	137.1	11.7	
WR-TWU-LF	GR-LX-WI	22	0.45	3.90	0.85	0.452	44.2	35.7	55.1	43.9	
Global mean		**23.2**	**0.30**	**4.04**	**0.87**	**0.481**	**31.2**	**24.6**	**88.3**	**15.8**	**39.6**
Global SD		**6.6**	**0.17**	**0.62**	**0.07**	**0.038**	**36.3**	**15.7**	**38.2**	**10.1**	**29.0**

All subpopulations with a proportional assignment > 0.20 are regarded as contributors to an admixed site. Admixtures with * indicate cases where only one subpopulation had an assignment > 0.20, but that assignment was > 0.75. Rare alleles per plant, alleles per locus, and expected heterozygosity are arithmetic means across loci. Distance to nearest dominated site excludes sites on the same farm, values are given in km. Within-subpopulation distance is the arithmetic mean of distances (in km) from a site to all other farms with the same dominant subpopulation, using the nearest site for farms with multiple sites. Mean and standard deviation of this metric were calculated for farms, not sites.

Appendix 2.

Evaluation of SSR markers used for this study

Conceptually, SSR markers function as quantitative, objective measures of genetic characters, which should be extremely replicable and portable. In practice, there is an unavoidable qualitative aspect to SSR scoring that potentially hinders the portability of SSR-based assays. Studies involving SSRs typically present only scored data, while the properties and dynamics of the individual loci are unreported. Thus, while initial SSR marker discovery is conducted in a spirit of collaboration for the benefit of the research community, the process of identifying and characterizing markers with actual uses for research is typically carried out in parallel by individual researchers. Developing a working set of markers for a specific organism is a non-trivial task, one which may discourage or limit research using the technology.

In an attempt both to contribute to the working knowledge of SSR markers for meadow fescue, and to increase the commensurability of this work with future research, we present an evaluation of the markers reported in Chapter 1 of this work, along with our observations of their behavior and scoring challenges they presented. Observed size ranges, fluorescent tags, and groups with successful multiplexing are also reported. In addition, we briefly describe the behavior of these markers with non-meadow fescue genetic material.

To reduce the cost of the assay, we multiplexed markers to the extent that was possible. We developed four mixes where markers with overlapping size ranges could be given different dyes. In mixes 1 and 3, all primer pairs could be amplified in a single reaction. Mixes 2 and 4 had to be separated into two distinct multiplexes (denoted by letters)

Table A2-1. Compatibilities and scoring challenges of SSR markers in this study

Marker	Label	Mix	Repeat	Size range	Peak Type	Notes
NFA059	TAMRA	1	(AG)15	132-142	Single	
NFA071	FAM	1	$(GA)_{10}$	159-178	Single	1
NFA112*	FAM	1	(GGTT)6	231-261	Single	1
NFA136	HEX	1	(TGTT)9	208-220	Double	
LM26	HEX	2-A	$(GT)_{15}(GCGT)_5(GT)_{11}$	201-224	Shoulder	1
NFA032	FAM	2-A	(CTG)6	354-360	Single	
NFA060	TAMRA	2-A	(TAG)8	212-221	Single	2
NFA099	HEX	2-A	(CCTCTt)4	312-372	Echo	3
B5E1*	FAM	2-B	$(CA)_3(CA)_5$	†	†	
LM29*	HEX	2-B	$(GT)_5$	99-102	Single/Double	4
M4213	FAM	2-B	$(GT)_8$AGGT	135-143	Single	
B1A8	FAM	3	$(TG)_7$	277-301	Comb	
DLF008	HEX	3	$(ACT)_7$	242-255	Single	1
LM15	HEX	3	$(GT)_5$	141-161	Single	5
NFA022	FAM	3	$(AGG)_6$	192-195	Single	
NFA027	TAMRA	3	$(TCT)_6$	172-219	Single	
NFA041	HEX	3	$(CTGAT)_4$	195-207	Shoulder	
PR3	FAM	3	$(CA)_{22}$	127-144	Single	
LMgSSR 02-05A	TAMRA	4-A	$(AC)_{12}$	170-185	Shoulder	
NFA064*	HEX	4-A	$(GAG)_8$	168-206	Single	2,6
Uni001	FAM	4-A	$(AC)_{17}$	102-180	Single/Comb	2, 3,7
LpSSR112	HEX	4-B	$(CA)_{20}$	234-237	Single	1
NFA048	FAM	4-B	$(CGC)_7$	272-281	Single	
NFA073	TAMRA	4-B	$(CCA)_6$	241-244	Single	

Notes: 1) one or more bins had adjacent borders, leading to ambiguity scoring between the two, 2) in some individuals, peak strength varied sufficiently to cause ambiguity in scoring, 3) false peak within the marker's range, falls outside of the regular bins and does not vary among individuals, 4) presence of a null allele was detected by frequent failure to produce a signal and by extreme rarity of heterozygotic individuals, 5) one allele was separated from others in this locus by a substantial size range, 6) some individuals had more than two peaks, but segregation did not provide evidence of two loci, 7) peak shape changes across the spectrum of allele size.

* Markers were assayed but not included in the study.

†We found peaks at 161 and 217 bp, which present in all samples but varied greatly in intensity relative to one another.

which were pooled for visualization. Four markers (B5E1, LM29, NFA064, NFA112) were excluded from the final analysis due to scoring difficulties. LM29 functioned perfectly, but could not be treated as a codominant marker due to the null allele. Both NFA064 and NFA112 produced 1, 2, or 4 peaks. The absence of 3 peak individuals, as well as the relatively high number of homozygous and 2 allele heterozygous individuals provided evidence that only two gene copies were involved, raising the possibility that certain alleles could somehow combine to form multiple peaks. Lacking a clear mechanism or a consistent model for behavior at this locus, we excluded these genes markers. In our hands, the B5E1 produced a single strong peak and multiple peaks too weak to score consistently. We expect this marker may be monomorphic in our study population.

Examples of the peak types described in Table A2-1 are given in Figures A2-1 to A2-4. Both single and double peaks (Figure A2-1) are straightforward to score, provided that bins are sufficiently separated from one another. The echo pattern (Figure A2-2) consists of secondary peaks appearing a set distance from primary peaks only in heterozygous individuals (Figure A2-2, bottom). Figure A2-2 also illustrates a false peak. Shoulder peaks have a substantial secondary peak, which varies in strength relative to the primary peak, while comb peaks have multiple such (Figure A2-3). In both cases, homozygous and insufficiently separated heterozygous individuals can be difficult to distinguish, while separated heterozygous alleles provide the most confidence for verifying alleles. The Uni001 was unusual in that its peak characteristics changed for different allele sizes, beginning with single peaks (Figure A2-4A) at the smallest sizes, developing a shoulder in the middle range (Figure A2-4B), and a comb-like structure in the largest alleles (Figure A2-4C). Note the structural pattern is independent of other alleles present (Figure A2-4D).

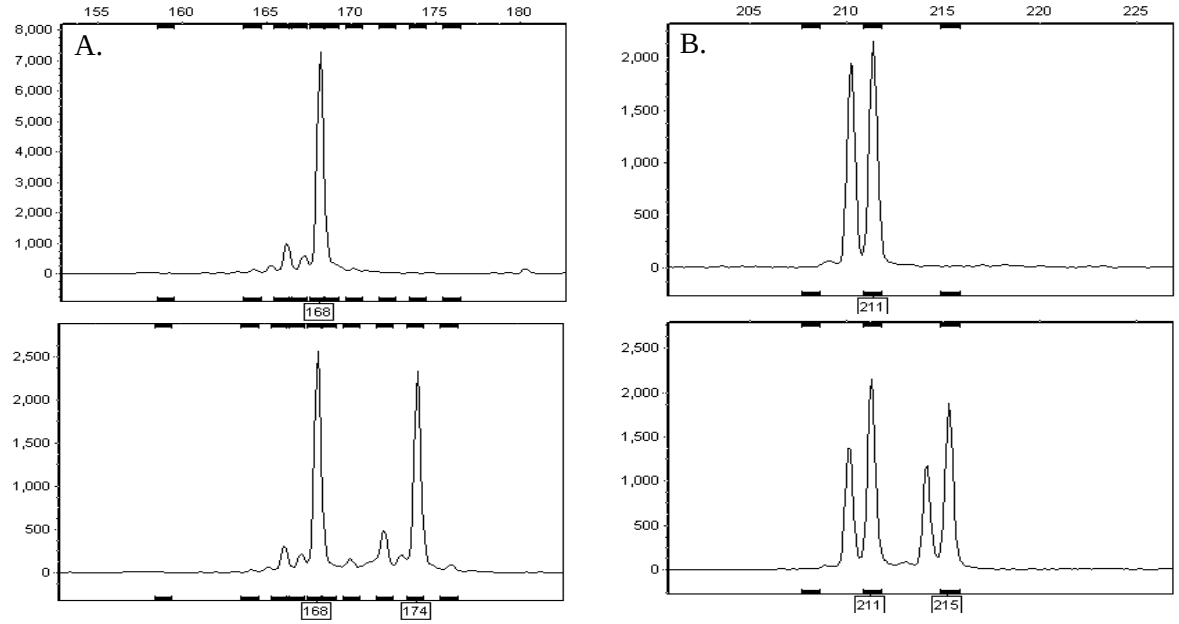

Figure A2-1. Simple peak patterns: A) simple peaks in NFA071, B) double peaks in NFA136. In both cases, top graph is homozygous, bottom graph is heterozygous.

Single peaks are from the NFA071 marker, double peaks are from NFA136. Sizes are given for true peaks

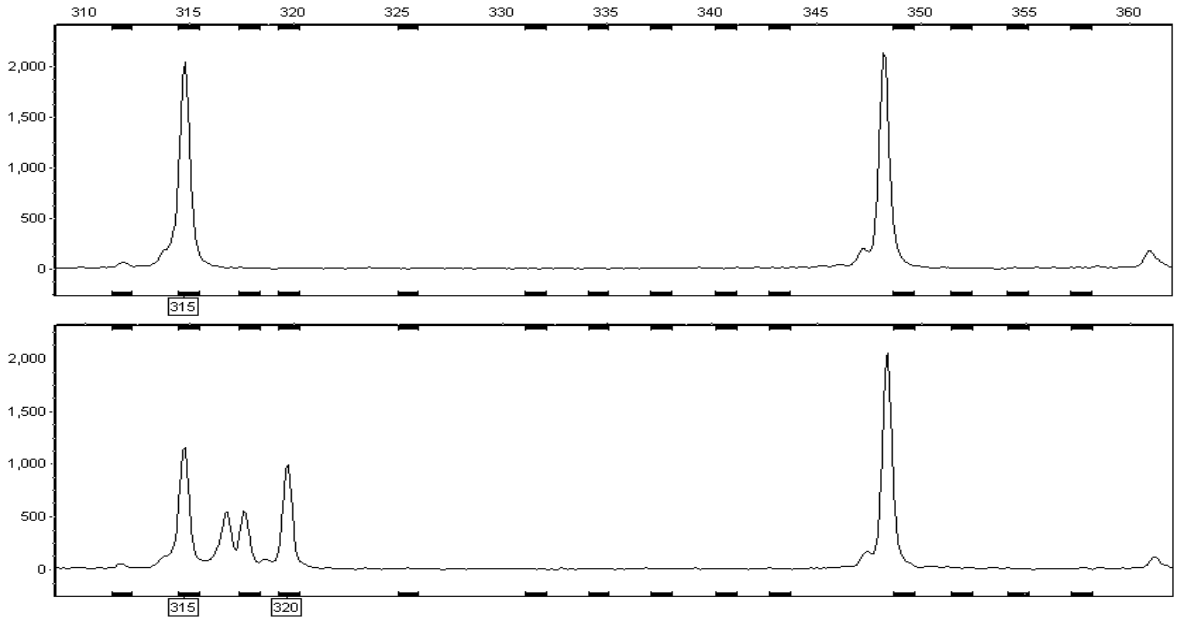

Figure A2-2. Echo peak pattern in NFA099. Top graph is homozygous, bottom is heterozygous. Peak at 349 is a false peak.

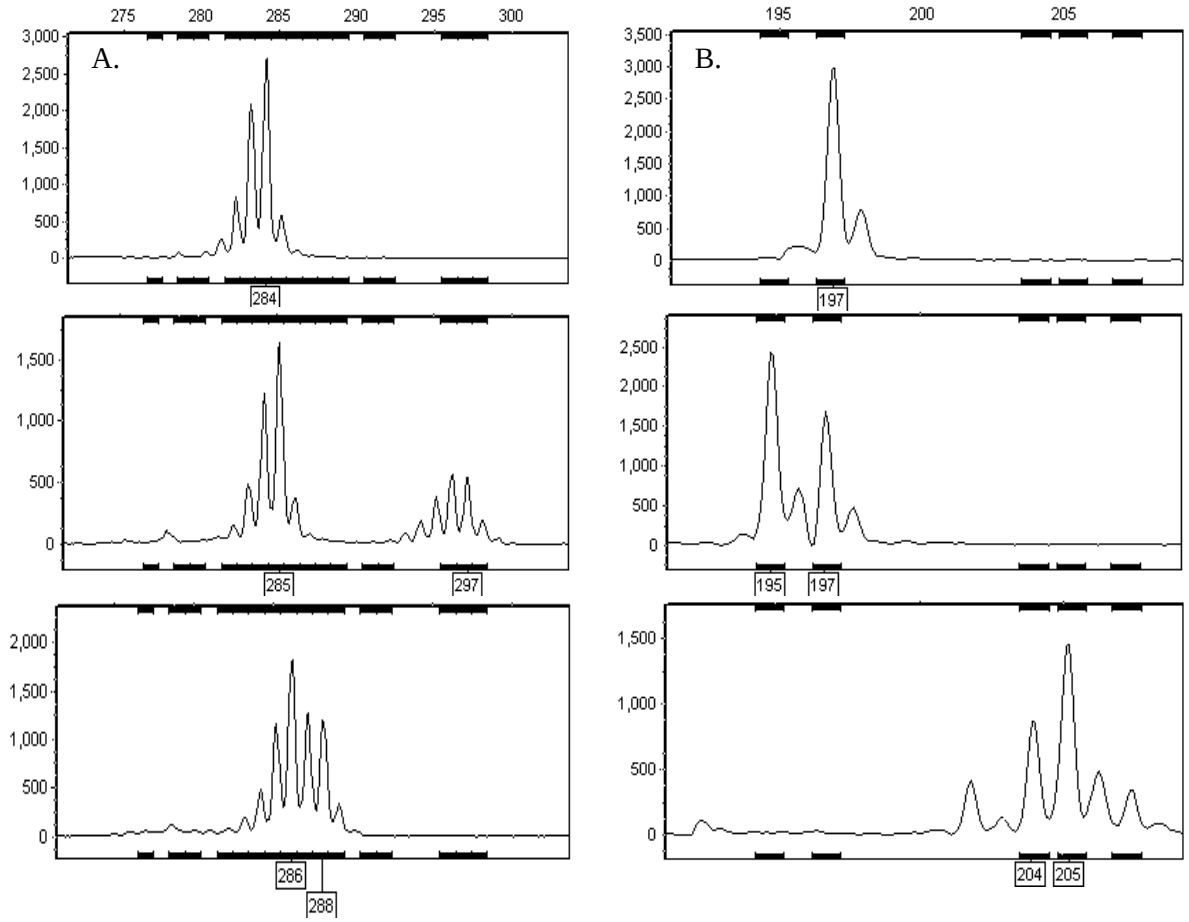

Figure A2-3. Complex peak patterns: A) comb pattern in B1A8, B) shoulder pattern in NFA041. Top panel is homozygous, middle and bottom are heterozygous. Sizes are listed for scored peaks. Note appearance of echo peaks in bottom panel of B.

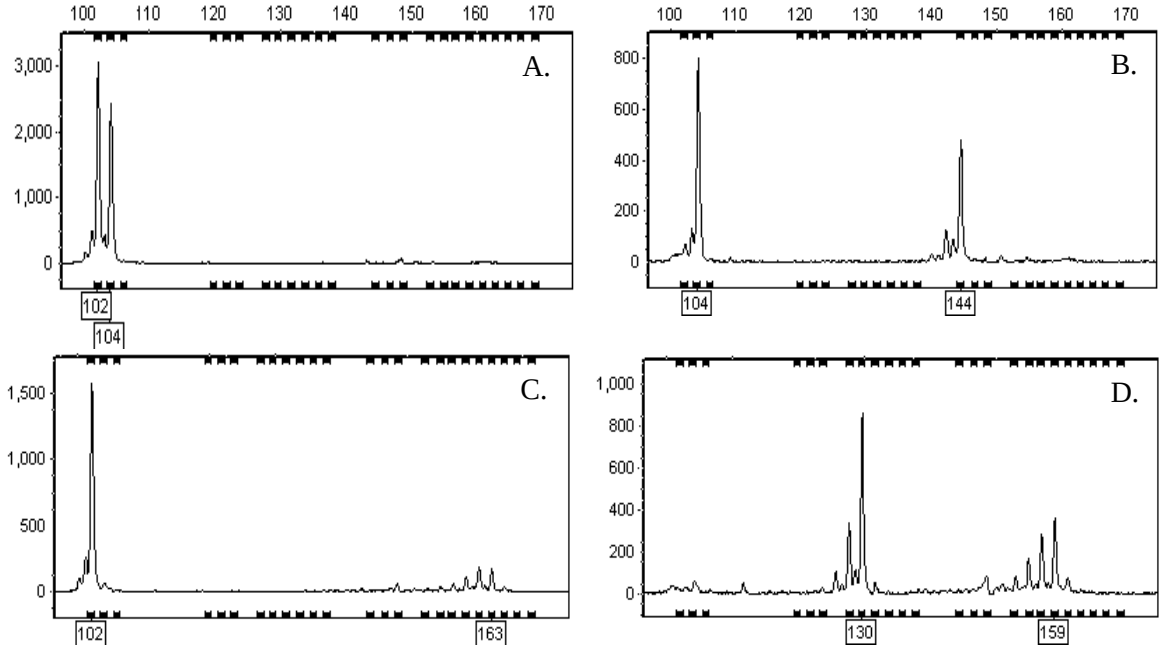

Figure A2-4. Changing peak structure in Uni001 marker: A) small, B) small and medium, C) small and large, D) medium and small alleles. All individuals are heterozygous. Sizes are given for marked peaks.

Due to morphological similarity among species in the *Festuca-Lollium* complex, we inadvertently collected multiple individuals that were not meadow fescue. While some of these were identified in the haplotype A screen or by sequencing during haplotype verification (Appendix 3), most were identified through their atypical patterns at SSR loci (Figure A2-5). Non-meadow fescue individuals typically contained more than two bands, either outside (Figure A2-5A) or within (Figure A2-5B) the normal range for allele in that marker. In some cases, a non-meadow fescue plant would contain multiple, oddly-sized bands in addition to valid alleles for a locus (Figure A2-5C). In this case, out of range alleles can be difficult to detect, as scoring software will typically report the valid alleles and neglect any bands outside of the range of established bins. We speculate these patterns may arise in

tall fescue or festulolium plants, which would contain both the meadow fescue locus and its analog from a closely related species.

Because we ran several markers in the same reaction, we could identify plants with abnormal patterns across multiple loci. Basing the decision to discard a sample on behavior at a single locus could lead to discrimination against plants with rare alleles, but individuals that display unusual behavior across numerous loci, particularly if the banding pattern suggests multiple gene copies, are more likely to truly be a different species. Moreover, not all species behave abnormally at all markers, making a multiplex approach essential to limiting identification errors. We initially tested all of our plants with the Mix 1 markers and used those results to screen for non-meadow fescue individuals. As the markers in this mix all produce simple banding patterns, it was easier to detect abnormal behaviors. Also worth noting is that the NFA112 marker, although not useful for determining meadow fescue genotype, contributed to identifying several non-meadow fescue plants.

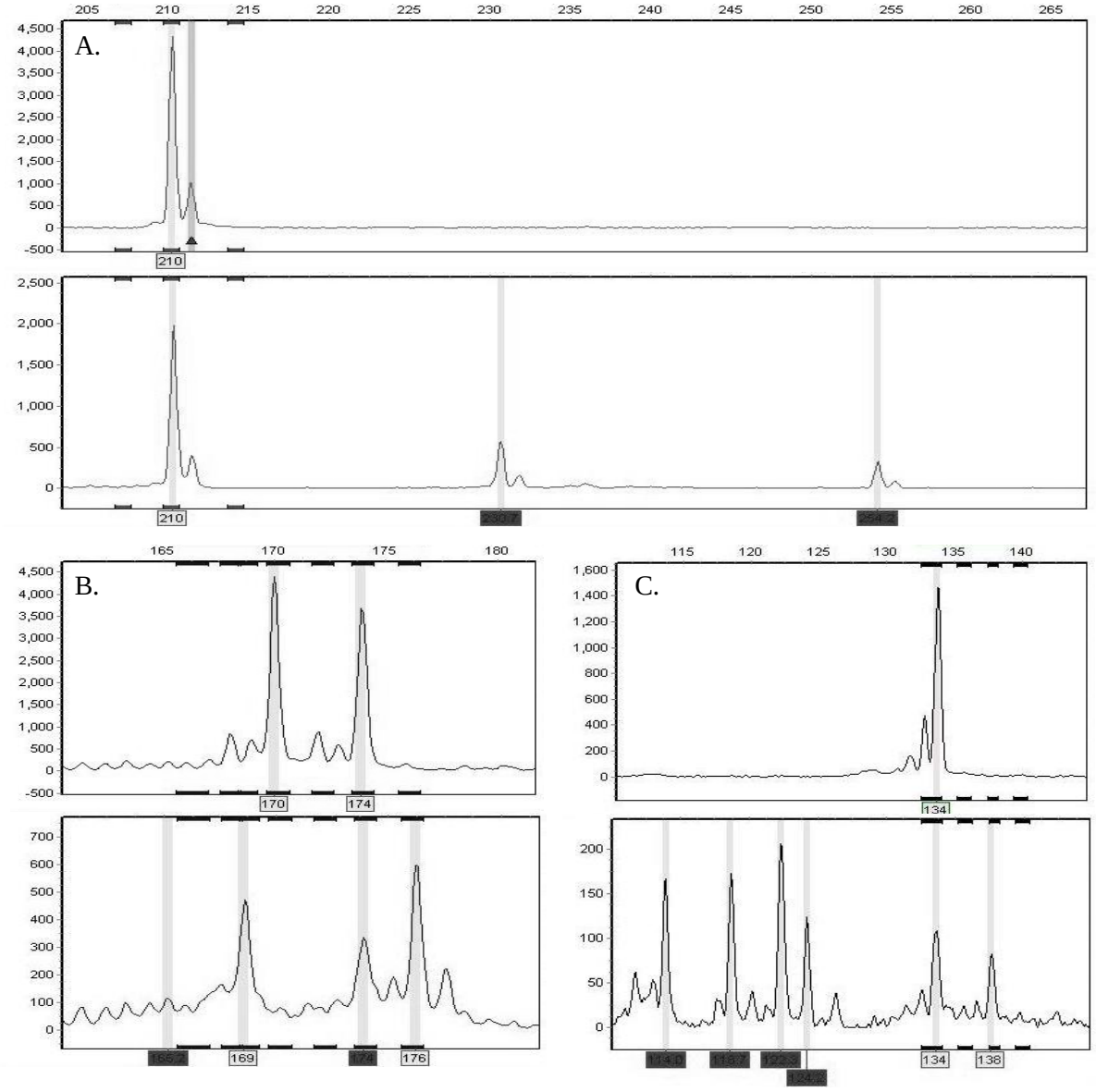

Figure A2-5. Diagnostic band patterns in non-meadow fescue plants. A) Multiple bands outside of the expected range, B) multiple bands within the expected range C) out of range bands with valid in-range alleles. Peaks called by the software are highlighted in grey, those with sizes in white were considered real, those with sizes in dark grey were flagged by the software.

Appendix 3.

Haplotype scoring assays

Sequencing conditions

Sequencing of psbD-trnS and trnT2-rps4 regions was conducted with nested primers from Fjellheim *et al*. (2006). Template amplification of cpDNA for sequencing used the JumpStart DNA amplification system (Sigma) with a program consisting of 35 cycles with annealing at 50 ºC for 60 s and extension at 65 ºC for 240 s, with a ramp to extension temperature of 0.3 ºC/s and extension temperature increasing by 0.2 ºC per cycle. Labeled product synthesis used the BigDye Terminator v. 3.1 system (Applied Biosystems), following the cycling protocol of Platt *et al*. (2007). Samples were electrophoresed on an Applied Biosystems 3730xl automated DNA sequencing instrument, using 50-cm capillary arrays and POP-7 polymer. All electrophoresis was conducted by the University of Wisconsin Biotechnology Center DNA Synthesis and Sequencing Facility. Consensus sequences for individual plants were constructed with the shotgun assembly module of Pregap4 v 1.5 and Gap v 4.10 from the Staden package based on double coverage, two-directional reads (Staden *et al.*, 2000). Sequence alignment was carried out in BioEdit (Hall, 1999) using the CLUSTAL W application (Thompson *et al.*, 1994).

MFhapA1

The A haplotype was assayed for an A to T transformation in the trS-psbD region that creates a *PsiI* restriction site in haplotype A individuals. A psbD-trnS subsegment was amplified using JumpStart, with a program consisting of 35 cycles of annealing at 50 ºC for 30 s and extension at 72 ºC for 60 s, using primers described in Table A3-1. The resulting

product was digested with *PsiI* (New England Biolabs) at a concentration of 0.1U enzyme for 5 uL product in NEB2 buffer at 37 C for 2 hours.

Table A3-1. Primer sequences and amplicon size

Primer	**Sequence (5'-3')**	**Amplicon size (bp)**
hapCR	CTATGCGAACCCCCTCTCTT	154
hapCF	AGAGATGGTGCGATTTGACTC	
hapAF	GAACCCTTTTTGCTTTCTTGA	87/88/94
habAR	ATATCGGCCGAGAGACAATC	

The amplified region is 154 long and contains one *PsiI* restriction site common to all haplotypes in addition to the one unique to haplotype A (Figure A3-1). After digestion, haplotype B/C/D individuals have 89- and 65-bp bands, while haplotype A individuals have 66-, 65- and 23 bp bands. For reasons that are unclear, when the product of digestion is run on a gel A haplotype individuals retain a faint band at 89 bp, but there is a clear shift in intensity toward the combined lower band (Figure A3-2A). In non-meadow fescue individuals, the amplification is successful, but the subsequent digest produces two bands that are clearly distinguished from the meadow fescue bands (Figure A3-2B). Effectiveness was verified by testing 4 individuals each of haplotypes A and B (determined by sequencing), and confirmed by sequencing 8 previously unidentified individuals assigned to each haplotype.

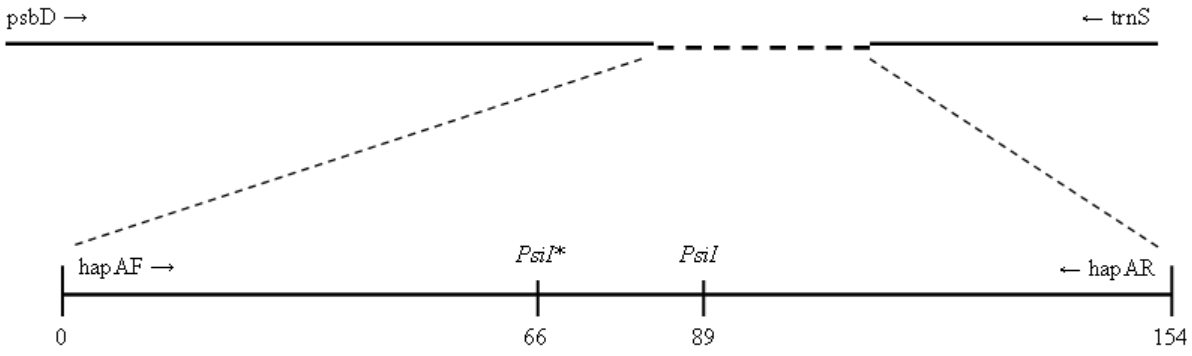

Figure A3-1. Diagram of psbD-trnS subsegment used for MFhapA1 assay. Numbers indicate basepairs from beginning of forward primer.

* indicates haplotype A-specific restriction site.

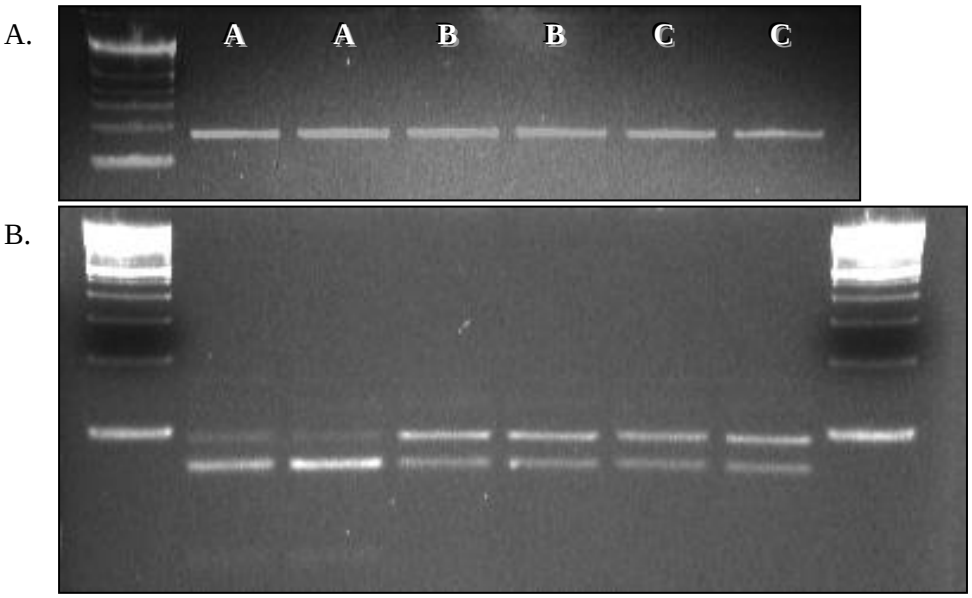

Figure A3-2. Banding patterns in MFhapA1 segment A) before and B) after P*siI* digest. White letters indicate haplotype of source DNA. Pictured are 100 bp ladders. Gels were run on 2% agarose in 1x SB.

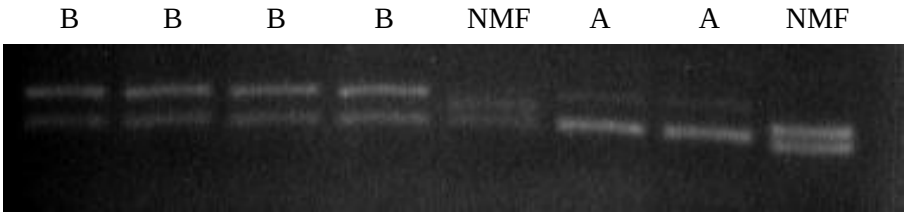

Figure A3-3. Banding patterns in MFhapA1 segment with non-meadow fescue (NMF) source DNA. Samples run on 2% agarose in 1x SB.

Haplotypes C and D

The haplotype C assay detects a 6 bp insertion in the trnT2-rps4 region in haplotype C individuals. We designed primers amplifying the segment of this region that contains the insertion (Table A3-1), and amplified this segment as part of the Mix 1 SSR multiplex (Appendix 2). On a capillary, this product was sized at 93 bp in haplotype C individuals and 87 bp in most non-haplotype C individuals (Table A3-2). We also discovered a second polymorphism in this region, producing an 88 bp product. We sequenced the entire region and identified a 1 bp deletion in an 8 A repeat that begins 6 bp after the haplotype C insertion (Table A3-2). In continuing the naming convention established by Fjellheim *et al.* (2006), we refer to the haplotype identified by this marker as haplotype D.

This marker also distinguished many non-meadow fescue individuals, as samples with unusual SSR marker patterns (see Appendix 2 for discussion) frequently produced 82, 86, or 88 bp bands for this locus.

Table A3-2. Haplotypic polymorphisms in the trnT2-rps4 chloroplast region

Haplotype	Sequence	Amplicon Length
C	GAAGACTGAGGTATAGGTA**TAGGTA**GGGCT**A**AAAAAAACA	94
A/B	GAAGACTGAGGTATAGGTA------GGGCT**A**AAAAAAACA	88
D	GAAGACTGAGGTATAGGTA------GGGCT-AAAAAAACA	87

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Appendix 4.

Meadow fescue habitat and communities in the Driftless Area

Methods

Study area and site selection

The study area for this study is described in Chapter 1 of this thesis. Study sites were identified in the summers from 2006 to 2008, typically by visual identification from the road, but occasionally by personal communication from farmers, and selected primarily to be representative of the location for genetic analysis. Sites 1-17 were identified in 2006, 18-88 in 2007, and 100-112 in 2008. Species and environmental data were collected either at recorded coordinates from plant sample collections (sites 1-88) or concurrently with sampling (100-112). There were 93 sites included in this survey, with 70 sites in common with the genetic survey and one site (103) with plant samples, but no composition or environmental data recorded. Species composition and soil samples were taken from June to July of 2008.

Population sampling

Percent plant basal cover was estimated using a grid-quadrat frame (Bonham, 1989). There were 5 measurements per site, with a central quadrat and 4 quadrats 2.4 meters away along cardinal directions. Each quadrat measured 1 meter on a side and contained 25 evenly spaced points along a grid with 0.25-m spacing, giving a total of 125 points covering 5 m^2 within a 3.4-m radius. Sites varied in their recent grazing/defoliation history, so composition was recorded at the soil surface, rather than from the canopy. Identification was carried to the species level for common pasture species, with coarser taxonomic level for groups with

lower frequency and less morphological differentiation (e.g. sedges and plantains). Mature trees were not considered in this study, and where necessary quadrats were relocated to avoid intersecting trees, but smaller woody species and seedlings were counted when encountered. Percent abundance was calculated in aggregate for all quadrats at a site.

Environmental measurements

Slope was measured as the maximum proportional change in elevation over 6 horizontal meters centered on the central quadrat. Aspect was recorded as one of 8 cardinal directions, then converted to continuous northness and westness by cosine and sine transformation respectively. Degree of shading was estimated visually and assigned to one of 5 categories: full sun, single tree shade, open canopy, forest edge, or full shade. At each site, land use was described in general terms (hay, pasture, fallow) based on personal communication or evidence of recent animal presence or mechanical management. Visually adjacent land cover elements (buildings, roads, pastures, row crops, trees, water, etc) were also noted (Table A4-2).

Soil analysis

For each quadrat, 4 soil probe samples were taken and immediately pooled. Soil cores were taken to a depth of 20 cm, or until an impassable restrictive layer was encountered. Soil samples were placed in airtight bags, refrigerated at 4 ºC within 10 hours of collection from the field and were either frozen at -20 ºC or sieved to 8 mm within 2 weeks of collection. Frozen soils were sieved immediately after thawing, and all sieved soils were refrozen. Sieved soils were thawed for 24 hours at 4 ºC prior to pH analysis. Soil pH was measured with a combined reference glass electrode in a 1:2 (wet weight/volume) suspension of soil in microfiltered water, following Robertson *et al.*, (1999). Immediately after pH testing, soils

were dried for 48 hours at 65 ºC and equal weights from each site replicate were combined and homogenized by repeated sieving to produce a single pooled sample for each site.

Soil texture was determined for pooled samples by quantifying soil sedimentation from a sodium-hexametaphosphate solution with a hydrometer. Percentages of total carbon and total nitrogen in the soil were determined by combustion on an FLASH EA 1112 NC Analyzer (CE Instruments). Soil samples for combustion were 8 - 10 mg and were combusted in pressed tin capsules. Triplicate samples were run for each site; the most extreme value was discarded from sites with a relative error greater than 7.5%. Lime requirement is based on Sikora buffer pH (Sikora, 2006), using equations from Laboski *et al*. (2006). Percent organic matter was determined by loss on ignition (Shulte, Hopkins, 1996). Phosphorus and potassium concentrations were determined using Bray's reagent and colorimetry or Atomic Absorption, respectively (Bray, Kurtz, 1945). Soil density, organic matter, P, K, and liming requirement values were determined by the UW-Madison Soil and Plant Analysis Laboratory.

Statistics

Site values for pH, percent carbon and nitrogen were arithmetic means of multiple measurements. Means reported in Tables A4-1 and A4-4 are arithmetic means for site values. Shannon's diversity index for a site is calculated as $SHDI_j = -S p_{ij} \ln(p_{ij})$, where p_{ij} is the proportional cover of the i^{th} species at the j^{th} site. Shannon's evenness index was calculated as $SHEI_j = SHDIj/\ln(Nj)$ where Nj is the species richness at the j^{th} site. The R package (version 2.9.0) was used to calculate correlations among parameters.

Data Tables

Table A4-1. Summary of site environmental characteristics

Parameter	Mean	SD	Min	Max	Units and interpretation
Latitude	42.88	0.28	42.56	43.81	Decimal degrees north
Longitude	90.31	0.38	89.94	91.51	Decimal degrees west
Slope	7.6	8.6	0.0 (29)	35.0	Percent, see materials and methods
Westness	0.04	0.53	-1.00	1.00	See materials and methods
Northness	0.13	0.56	-1.00	1.00	See materials and methods
pH	6.9	0.6	5.3	7.8	
Sand	22.6	12.4	10.8	67.6	Percent by weight
Clay	24.4	6.4	7.0	41.0	Percent by weight
OM	5.0	2.2	2.0	15.9	Percent soil organic matter by weight
P	40.2	36.8	6.0	221	Total soil phosphorus, ppm
K	121.0	83.5	29.0	527	Total soil potassium, ppm
Lime	1.5	3.6	0.0 (65)	19.4	Tons of lime per acre required to lower pH to 6.6
Density	0.90	0.10	0.72	1.21	Grams of dry ground soil per cm^3
N	0.28	0.13	0.09	1.11	Total soil nitrogen, percentage by weight, 0.85 correlated to C
C	3.37	1.79	1.15	13.81	Total soil carbon, percentage by weight
C/N	11.96	2.91	7.89	31.20	Ratio of carbon to nitrogen in the soil

Correlation between N and C was 0.93.

Table A4-2. Frequency and criteria for landscape elements

Element	Element frequency	Criterion
2006	0.18	Sites on a single farm where meadow fescue was intentionally spread, see comments
Shade	0.32	Whether site sampled was under shade of any kind.
Pasture (use)	0.89	Whether site sampled was used as pasture during the sampling year.
Hay (use)	0.09	Whether site sampled was used for hay production during the sampling year, not exclusive with use as pasture.
Buildings	0.34	Whether buildings of any kind were visibly adjacent to the field sampled.
Row Crops	0.39	Whether fields planted with annual row crops were visibly adjacent to the field sampled.
Hay (adjacent)	0.23	Whether fields grown for hay were visibly adjacent to the field sampled.
Pasture (adjacent)	0.92	Whether other fields used for pasture were visibly adjacent to the field sampled.
Road	0.35	Whether paved roads were visibly adjacent to the field sampled.
Trees	0.68	Whether mature trees were within or visibly adjacent to the field sampled.
Water	0.40	Whether surface water was within or visibly adjacent to the field sampled.

The 2006 element describes the 17 sites that were sampled that year. Relative abundance of meadow fescue was extremely high at these sites (mean 0.680 compared to mean 0.320 for all other sites, *t* test $p < 0.001$). Use as pasture was the only parameter to have a significant predictive value for meadow fescue abundance, being negatively correlated with meadow fescue abundance ($p = 0.035$). However, only 6 sites were not classified as used for pasture, making generalizability dubious.

Table A4-3. Global abundance and presence for common species

Group	Common name	WISFLORA nomenclature	Percent cover over all sites	Number of sites present
Grasses	meadow fescue	*Festuca pratensis* Huds.	38.2	90
	Kentucky bluegrass	*Poa pratensis* L.	17.1	84
	quackgrass	*Elytrigia repens* (L.) Desv. ex B.D.Jacks.	8.1	76
	timothy	*Phelum pretense* L.	7.6	66
	tall fescue	*Festuca arundinacea* Schreb.	5.7	27
	orchardgrass	*Dactylis glomerata* L.	4.3	47
	redtop	*Agrostis gigantea* Roth	2.1	13
	smooth brome	*Bromus inermis* Leyss	1.6	20
Legumes	white clover	*Trifolium repens* L.	3.9	52
	red clover	*Trifolium pretense* L.	1.4	30
	crown-vetch	*Coronilla varia* L.	1.1	3
Forbs	dandelion	*Taraxacum officinale* Weber	2.5	58
	wild carrot	*Daucus carota* L.	0.9	21
	creeping-Charlie	*Glechoma hederacea* L.	0.6	23
	thistle	*Cisrium* spp	0.6	5

These 13 species represent 95.6% of the plant cover throughout the study area. Crown-vetch was found only in three sites (all in the 2006 group), in what were essentially crown-vetch hay fields, causing its abundance in the area to be exaggerated. Approximately half of the tall fescue identified came from 5 sites with little or no meadow fescue, where tall fescue was initially misidentified as meadow fescue. Tall and meadow fescue co-occur at much lower rates than these data suggest.

Table A4-4. Summary of species abundance and diversity

	mean	SD	max	min	2006 *P*-value	
MF	0.386	0.236	0.904	0.000	<.001	Proportion of meadow fescue cover
Legume	0.068	0.103	0.443	0.000	0.187	Proportion of legume cover
SR	8.4	2.9	16	4	0.038	Number of species per site
GSR	4.9	1.3	9	2	0.011	Number of grass species per site
SHDI	1.411	0.405	2.314	0.395	< .001	Shannon's diversity index
SHEI	0.678	0.141	0.914	0.254	<.001	Shannon’s evenness index

Legumes included red and white clover, crown vetch, and alfalfa (*Medicago sativa* L.). All metrics served as highly significantly negative predictors of meadow fescue abundance (all $p < 0.001$). *P*-values are for unpaired *t*-tests between 2006 sites and all other sites.

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