

Potential selection in native grass populations by exotic invasion

BRIAN A. MEALOR and ANN L. HILD

Department of Renewable Resources, University of Wyoming, Box 3354, Laramie, Wyoming 82071, USA

Abstract

Ecological impacts of invasive plant species are well documented, but the genetic response of native species to invasive dominance has been often overlooked. Invasive plants can drastically alter site conditions where they reach dominance, potentially exerting novel selective pressures on persistent native plant populations. Do native plant populations in old exotic invasions show evidence of selection when compared to conspecific populations in adjacent, noninvaded areas? We employ amplified fragment length polymorphism (AFLP) analysis to screen a large number of loci from two native grass species (*Hesperostipa comata* (Trin. & Rupr.) Barkworth and *Sporobolus airoides* Torr.) that occur in old infestations of the invasive forb *Acroptilon repens*. We then compare observed locus by locus F_{ST} values with distributions of F_{ST} estimated from simulation models under expectation of neutrality. We also compare the proportion of loci possibly linked to selection and those not linked to selection which exhibit parallel trends in divergence between two community types (invaded, noninvaded). Few loci (*H. comata*, 2.6%; *S. airoides*, 8.7%) in the two native grasses may be linked to genes under the influence of selection. Also, loci linked to selection showed a greater portion of parallel trends in divergence than neutral loci. Genetic similarities between community types were less than genetic similarity within community types suggesting differentiation in response to community alteration. These results indicate that a small portion of scored AFLP loci may be linked to genes undergoing selection tied to community dominance by an invasive species. We propose that native plants in communities dominated by exotic invasives may be undergoing natural selection.

Keywords: AFLP, genetic differentiation, genomics, invasive species, native grasses, selection

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Introduction

Many ecological impacts of nonindigenous invasive plants have been well documented (D'Antonio & Vitousek 1992; D'Antonio & Meyerson 2002). However, seldom have researchers investigated the effects of community dominance by invasive plants on the genetic makeup of native plant populations (Mealor *et al.* 2004). Species invasions greatly alter physical and biotic environments (Mooney & Cleland 2001). Subsequently, the biological rearrangements of species invasions alter evolutionary trajectories both of those species which are introduced into new areas and of native species in recipient communities. Here, we examine supposedly altered evolutionary trajectories using molecular genetic markers.

Invasive species can rapidly evolve in areas of introduction via founder effects and hybridization (Vila & D'Antonio 1998; Ellstrand & Schierenbeck 2000; Leger & Rice 2003), and by responding to selective pressures associated with their new environment (Siemann & Rogers 2001; Maron *et al.* 2004). Adaptive evolution may provide a key mechanism allowing success of exotics in new neighbourhoods (Maron *et al.* 2004). Rapid selection of invasive species may be driven by a new abiotic environment, interactions with new neighbours, and release from natural enemies (Maron & Vila 2001; Cox 2004). The same suite of alterations should be associated with native plants that persist in communities dominated by invasive species. Persistent natives, rather than being introduced into a novel environment (as are invasives), have had novel conditions imposed on them. After an initial reduction of native plant abundance, remaining native plants in old invasions may represent a subset of individuals particularly well suited for coexistence with

Correspondence: Brian A. Mealor, Fax: 307 766 6403; E-mail: bamealor@uwyo.edu

exotic invaders (Mealor *et al.* 2004), and may show signs of natural selection for resistance to potentially toxic allelopathic chemicals produced by successful invaders (Callaway *et al.* 2005). Habitat variability can lead to genetic differentiation between local populations (Antonovics 1971; Linhart & Grant 1996). Plant populations may show rapid differentiation in response to contemporary changes in soil chemistry (Snaydon & Davies 1982; Al-Hiyaly *et al.* 1988), defoliation (Kölliker *et al.* 1998), and community characteristics (Odat *et al.* 2004). It may be possible to detect the signature of such rapid, localized selection using current molecular methodologies.

Invasive knapweed species in North America alter soil chemistry (Stevens 1986), increase soil erosion, and reduce forage quality and wildlife populations (Goslee *et al.* 2001). *Acroptilon repens* (L.) DC (Russian knapweed) is a highly competitive, herbaceous perennial. A native to Eurasia, it forms dense monocultures in disturbed areas (Watson 1980). The impacts of *A. repens* may be more detrimental than other knapweeds (*Centaurea* spp.) because of its clonal nature (Mullin *et al.* 2000). *A. repens* dominance has been partly attributed to effects of allelopathic chemicals produced in its roots and leaves (Stevens 1986), particularly in fine-textured soils (Goslee *et al.* 2001). Consequently, it may be asserted that long-standing knapweed infestations of native plant communities produce two distinct community types: native plant community and knapweed-dominated community, each characterized by a different suite of traits. Allelopathic chemicals found in soils beneath *A. repens* invasions may not be present in areas free of infestations (see Callaway & Aschehoug 2000; Bais *et al.* 2003). *A. repens* could be described as an ecosystem transformer (Richardson *et al.* 2000) transforming grasslands into communities dominated by knapweed. Given the ability of *A. repens* to alter site conditions, native plants that persist within dense stands of *A. repens* are subject to different selective pressures than conspecifics growing in areas free of the invader. Additionally, evolution in response to biotic forces (i.e. competition) represents a potentially never-ending arms race which is measured by continuing to play the game rather than by winning (Harper 1977). Because *A. repens* is novel to native grassland species, and does not share a long co-evolutionary history, native plants persisting inside infestations are subject to new biotic interactions. Although *A. repens* stands may appear monospecific, native grasses are present in old invasions (Mealor *et al.* 2004). It is reasonable to suggest that community transformation by an invasive species could affect the genetic composition of remaining native populations.

Multilocus markers can be used to separate locus-specific effects from genome-wide effects (Luikart *et al.* 2003). Of the methods available today, amplified fragment length polymorphisms (AFLPs; Vos *et al.* 1995) provide a practical and affordable means of screening large numbers of loci for nonmodel organisms. In this study, we investigate effects

of local selection on native grass populations in knapweed-dominated vs. noninvaded native communities. Using AFLPs, we assess genetic diversity and divergence of *Hesperostipa comata* (Trin. & Rupr.) Barkworth (Needle and thread) and *Sporobolus airoides* Torr. (Alkali sacaton) in the two communities. We employ the approach of Bowcock *et al.* 1991 and Beaumont & Nichols (1996) which uses simulations based on empirical data to develop an expected distribution of F_{ST} values under neutrality. We compare our observed and expected values to identify outlier (falls outside an expected distribution) loci which may have been acted upon by selection or are closely linked to loci under selection (Beaumont & Nichols 1996).

We assess patterns of genetic differentiation at AFLP loci between populations of native grasses in two community types (invaded by knapweed, noninvaded) to identify loci that are potentially linked to genes implicated in selection (S_L loci). Next, we determine whether S_L loci are indicative of parallel patterns of divergence at three geographical locations for each grass species. More specifically, we test whether: (i) AFLP loci demonstrate evidence of selection between invaded and noninvaded communities, (ii) similar patterns of parallel divergence occur at multiple locations, and (iii) divergence is greater between community types than within community type.

Materials and methods

Species and site descriptions

Two grass species were selected for study because they are commonly found in invasions of *Acroptilon repens* in the Intermountain West (Mealor *et al.* 2004). *Sporobolus airoides* Torr. (Alkali sacaton) is a warm season perennial bunchgrass widely distributed throughout western North America. This grass is useful for restoration practices because of its ability to tolerate saline soils (Aldon 1981) and ability to accumulate potentially toxic compounds in soils (Retana *et al.* 1993). After establishment, *S. airoides* is tolerant of both drought and inundation by water (Johnson 2000). In productive environments, *S. airoides* is more competitive than other arid rangeland grasses (Novoplansky & Goldberg 2001). *S. airoides* is predominantly a self-pollinator (Fryxell 1957) and therefore may be affected by localized selection with little impact from gene flow via pollen (Grant 1971).

Hesperostipa comata (Trin. & Rupr.) Barkworth (Needle and thread) is a cool season perennial bunchgrass useful for stabilizing degraded or eroded rangeland sites (Zlatnik 1999). *H. comata* tolerates drought (Mueller & Weaver 1942) and distributes most of its roots in the upper 0.2 m of soil (Melgoza & Nowak 1991). Although Freeman & Emlen (1995) assert that *H. comata* is a weak competitor with several rangeland species, it is found persisting in old *A. repens* invasions (Mealor *et al.* 2004). *H. comata* is capable of either self- or

Table 1 Descriptive characteristics of six *Acroptilon repens*-invaded rangeland sites

Grass species	Location	Altitude (m a.s.l.)	<i>A. repens</i> density (stems m ⁻²)	*Invasion age (year)	Soil (texture class)
<i>Hesperostipa comata</i>	Laramie, WY	2194	7	40	Sandy loam
	Mud Lake, ID	1460	80	70	Loamy sand
	Riverton, WY	1524	30	35	Sandy loam
<i>Sporobolus airoides</i>	Greybull, WY	1190	57	40	Silty clay
	Laramie, WY	2189	14	40	Silty clay loam
	Naturita, CO	1621	17	75	Loamy sand

Altitude in metres above sea level (m a.s.l.).

*Age of invasions is based on literature citations and herbarium specimens for each locale.

cross-pollination (Fryxell 1957), suggesting that divergence may be observed only under very strong selection (Grant 1971). Both grass species are potentially long-lived and exhibit a high degree of clonality. Although both are used in restoration efforts, we had no evidence that our rangeland locations had undergone restoration plantings in the past.

After extensive searches in Wyoming, Idaho, and Colorado, we identified three *A. repens* invasion locations for each native grass species. Sites were selected for three criteria: evidence of knapweed infestation for at least 25 years, a clearly visible boundary between invaded and noninvaded communities, and comparable soil textures between community types. Collection sites varied in age, altitude, and weed density (Table 1). Invasion age was based on herbarium records, publications of knapweed locations, and on incidences of drastic disturbance that may have contributed to knapweed invasion. For example, Rogers (1928) reports infestations of knapweed in southern Montrose County, Colorado as early as 1928 near our collection site near Naturita. Our Naturita collection site is located near the Montrose and San Miguel County border, and is tentatively aged at approximately 75 years. The earliest herbarium record of *A. repens* in Jefferson County, Idaho is from 1934 (invaders database). *A. repens* was reported in irrigated and occasionally in nonirrigated areas throughout Wyoming as early as 1935 (Sabin 1935), and was considered the most serious weed pest in Wyoming by 1940 (Boyd & Corkins 1940). The first herbarium record for northern Albany County and Big Horn County, Wyoming are 1950 and 1981, respectively. *A. repens* occurred near our Wyoming collection sites in Fremont, Big Horn and Albany counties by 1962 (Mitich *et al.* 1962). Recognizing that herbarium or other published records are often obtained later than the first occurrence of plants, these may not be the actual ages of *A. repens* infestations at our sites, but are the best available estimates. Geographical distances between locations ranged from 723 km (Greybull to Naturita) to 291 km (Laramie to Riverton).

Sample collection

We sampled hierarchically for each native species at three levels: geographical location, community within location and subpopulation within community. At each location, community types were described categorized as invaded or noninvaded by the density of fresh green *A. repens* stems (Table 1). Invaded communities contained high densities of *A. repens* (7–80 stems m⁻²) and the invasive was absent from noninvaded communities. Within each community type, we collected actively photosynthesizing leaf tissue from 10 to 15 individual grasses in each of two subpopulations (~30 individuals per community type per location). Community types and subpopulations within communities were separated by no less than 15 m and no more than 20 m (Fig. 1). By sampling mature plants rather than seed, we targeted those individuals able to persist inside invasions, but this strategy may not address current levels of gene flow between communities. Leaves were immediately placed in silica gel and stored in an ice-filled cooler for transportation. Samples were kept in a freezer at –20 °C at the University of Wyoming until DNA extraction using the standard 2× CTAB (cetyltrimethyl ammonium bromide) protocol (Doyle & Doyle 1987).

Molecular analyses

Amplified fragment length polymorphisms (AFLPs) were performed as described by Vos *et al.* (1995) except that *EcoRI* selective amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein) label on the 5' nucleotide. We screened over 40 selective primer combinations for the ability to consistently produce more than 40 total bands per native species. Of those screened, we chose five *EcoRI/MseI* primer pairs (ACG.CAC, ACG.CTA, ACG.CTC, ACGT.CTTC, AGT.CTT), to be used in selective amplifications as performed in Larson *et al.* (2004). Selective amplification profiles were compared to primer pair screening profiles

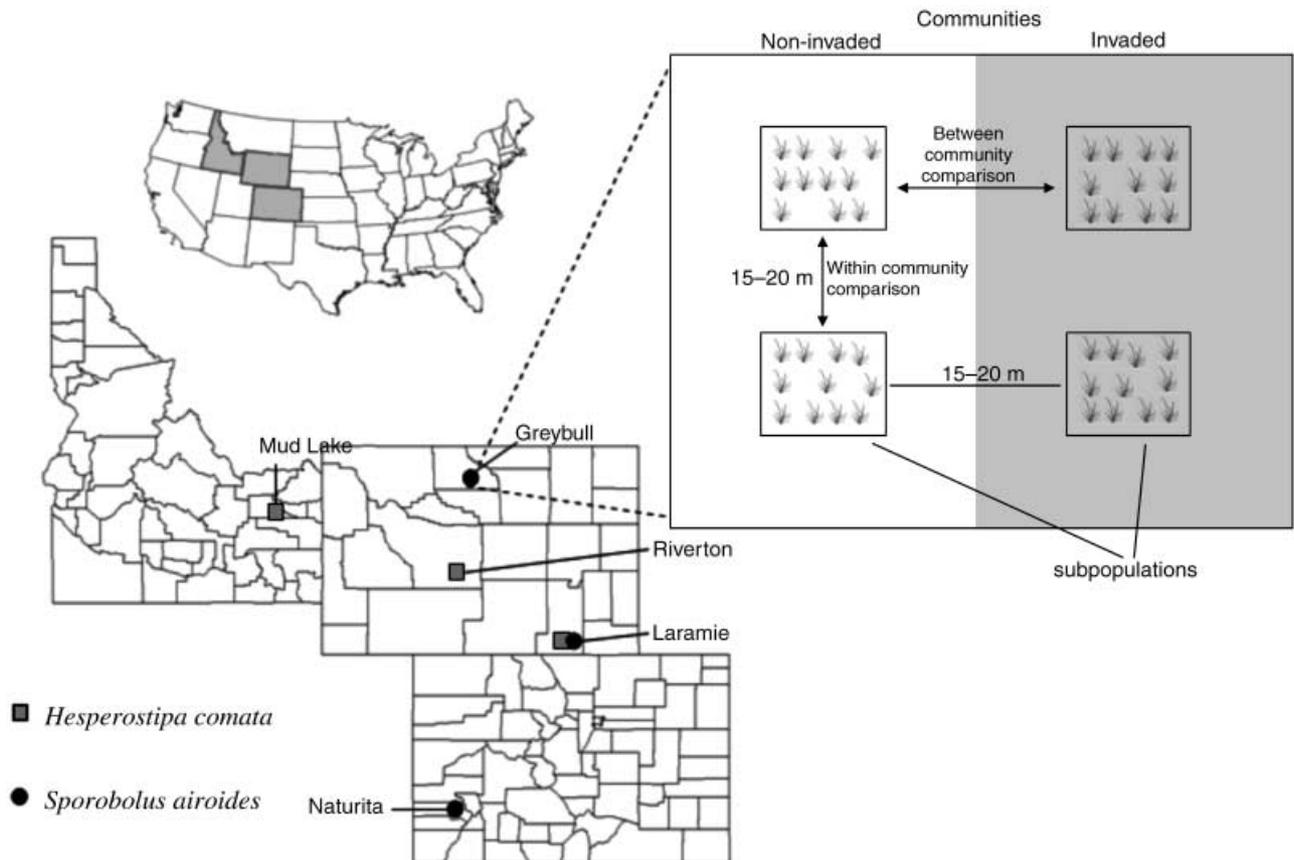


Fig. 1 Sampling scheme at each of six geographical locations invaded by *Acroptilon repens*. *Hesperostipa comata* collections were made near Mud Lake, Idaho, Laramie, and Riverton, Wyoming, whereas *Sporobolus airoides* collections were made near Naturita, Colorado, Greybull, and Laramie, Wyoming. Community types (noninvaded, invaded) were divided into subpopulations (small square plots) for further comparisons.

and showed a high degree of concordance. Amplified DNA fragments were size-fractionated using an ABI 3100 instrument (Applied Biosystems) with 50-cm capillaries, POP-6 Polymer, GENESCAN 400HD ROX (rhodamine X) internal size standards, and GENESCAN software (PE Applied Biosystems). The GENESCAN sample files were visually analysed for presence or absence of DNA fragments between 50 and 400 base pairs using GENOGRAPHER version 1.5 (Benham *et al.* 1999), which reduced subjective scoring of profiles. We considered a locus to be polymorphic if at least one individual showed a variant pattern.

Data analysis

Inference of loci under selection. To identify loci that may have been influenced by selection, we followed the approach developed by Bowcock *et al.* (1991) and Beaumont & Nichols (1996). We used the program *DFDIST*, kindly provided by Dr Mark Beaumont, to simulate the distribution of F_{ST}

related to heterozygosity across loci under neutral expectations. *DFDIST* differs from the distributed *FDIST* (Beaumont & Nichols 1996) and *FDIST2* (Beaumont & Balding 2004) in that it uses Zhivotovsky's (1999) approach to calculate the simulated values for heterozygosity and F_{ST} and it uses the overall heterozygosity of the pooled sample. The program uses coalescent simulations to generate data sets with a distribution of F_{ST} close to the empirical distribution and calculates approximate *P*-values for each sample locus (Beaumont & Balding 2004). For each grass species at each location, we grouped individuals according to community type and ran simulations to generate expected distributions of heterozygosity and F_{ST} . Loci with F_{ST} values above the upper 95% quantile for this simulation were inferred as being potentially under directional selection in accordance with community differences (hereafter selected loci, S_L). The 95% quantile represents a one-tailed hypothesis of linkage to areas of the genome potentially under selection (see Beaumont & Nichols 1996). We labelled loci with F_{ST}

estimates within the expected boundaries as not linked to genes under selection (NS_L).

Parallel patterns of divergence. Parallel trends in divergence suggest that observed divergence may have been affected by selection (Gravilets 1997; Campbell & Bernatchez 2004). Within each grass species, we describe trends in the divergence of S_L markers among the three locations to reveal parallel selection (Campbell & Bernatchez 2004). We designated a locus as having a parallel trend in divergence if its frequency of band presence in one community type was at least 5% greater than in the other community type at all three locations. For instance, if locus x had a higher frequency of band presence for the invaded community type in all three locations, then we would determine that locus x exhibits a parallel trend in divergence.

We used a χ^2 test to compare observed and expected proportions of NS_L and S_L loci showing parallel trends in divergence. To calculate expected proportions of loci exhibiting parallel trends, we used the product of the proportions of NS_L loci with parallel trends for both invaded and non-invaded community types over all three locations for each species (Campbell & Bernatchez 2004). We multiplied this expected proportion by the absolute number of loci in each category (NS_L , S_L) to assign expected values to be used in the chi-square test. For example, if locations A, B, and C had proportions of NS_L with parallel trends of 0.4, 0.3, and 0.5, respectively, then the product of those proportions would be 0.06. Thus, the expected proportion of loci to exhibit parallel trends in divergence would be 0.06. If selection is occurring between the two community types, we would expect to see an excess of observed parallel trends in S_L relative to NS_L loci.

Genetic similarity. We used populations to address whether observed patterns of divergence are greater between community types within a location than within-community types. We analysed S_L loci separately from NS_L loci. With dominant markers, such as AFLP, Hardy–Weinberg equilibrium is often assumed so that allele frequencies can be calculated for genetic analyses. This assumption could be easily violated with dominant markers (Culley & Wolfe 2001) and further complicated by potential self-fertilization in our study species. To avoid these complications, we used a measure of genetic distance that only uses bands themselves (Nei & Li 1979), without invoking Hardy–Weinberg equilibrium. Within each location, tests of population subdivision and comparisons of within-population similarity were based on the similarity coefficient (Leonard *et al.* 1999) for each possible pair of individuals, both within and between populations (Silbiger *et al.* 2001). To determine whether the populations show evidence of being genetically distinct, we tested population subdivision with 1000 random permutations resulting in a test P value

based on the ranking of the observed test statistic (relationship of within subpopulation similarity to overall similarity across subpopulations) relative to the set of relationships generated from the permuted data (Leonard *et al.* 1999; Silbiger *et al.* 2001). We report within population measures as similarity and between population measures as distance (1 – similarity). If selective pressures on outlier loci were driving divergence between community types, then we would expect to see greater genetic distance between community types in S_L than within community types in S_L . We also predicted that genetic distance would be similar within and between community types in selectively neutral loci, NS_L .

Results

Hesperostipa comata

Five AFLP primer combinations produced 454 loci (84.6% polymorphic). A range of 47–124 bands were observed per primer pair in 139 individual *Hesperostipa comata* plants (Table 2). There were no fragments exclusive to either invaded or noninvaded communities at any of the three locations. When we compared observed F_{ST} values to simulated F_{ST} values for polymorphic loci in *H. comata* separately for each location, 10 loci (2.6%) fell above the upper 95% quantile indicating potential linkage to genes under selection (Table 3). Number of S_L loci varied by location and by primer combination (Table 3).

Twenty-four loci (6.3%) exhibited parallel trends in divergence, 22 among NS_L and 2 among S_L loci. The proportions of greatest band presence in the invaded community were: Laramie (0.353), Mud Lake (0.310), and Riverton (0.283) (product = 0.031). The proportions of greatest band presence in the noninvaded communities was 0.294, 0.308, and 0.252 (product = 0.024), respectively. The proportion of loci expected to exhibit parallel trends in divergence, therefore was calculated as the sum of the products for each community type (0.030 + 0.024 = 0.054, or 5.4%). We observed no excess of parallel trends in NS_L loci ($\chi^2 = 0.189$, $P = 0.664$) whereas S_L loci showed a significant excess of parallel trends in divergence ($\chi^2 = 4.20$, $P = 0.040$, Table 5).

On a more local scale, we tested for differences in genetic distances within a community and between communities at each location. When using S_L loci, mean genetic similarity between communities was smaller (0.684) than mean similarity within communities (0.725, Table 4). Results for NS_L loci were comparable; similarity between communities was less (0.777) than within communities (0.820, Table 4).

Sporobolus airoides

AFLP analysis produced 553 loci (92.6% polymorphic) in 162 individual *Sporobolus airoides* plants. The number of bands

Table 2 Levels of polymorphism of scored AFLP loci. A locus was considered polymorphic if at least one individual showed a variant pattern

Species	Primer combination					Total No. of bands
	ACG.CAC	ACG.CTA	ACG.CTC	ACGT.CTTC	AGT.CTT	
<i>H. comata</i>						
Variable bands	41	99	55	97	92	384
Fixed bands	6	3	6	23	32	70
Total	47	102	61	120	124	454
<i>S. airoides</i>						
Variable bands	74	120	78	107	123	512
Fixed bands	2	6	6	7	20	41
Total	76	126	84	114	153	553

Table 3 Loci potentially linked to genes under selection (S_L) in two native grass species at three locations

Species Location	Number S_L	Primer combination				
		ACG.CAC	ACG.CTA	ACG.CTC	ACGT.CTTC	AGT.CTT
<i>H. comata</i>						
Laramie	4.0	1.0	0.0	0.0	3.0	0.0
Mud Lake	0.0	0.0	0.0	0.0	0.0	0.0
Riverton	6.0	0.0	3.0	2.0	0.0	1.0
Mean	3.0	0.3	1.0	0.7	1.0	0.3
<i>S. airoides</i>						
Greybull	14.0	2.0	3.0	2.0	0.0	7.0
Laramie	11.0	1.0	3.0	1.0	2.0	4.0
Naturita	21.0	2.0	5.0	4.0	6.0	4.0
Mean	15.3	1.7	3.7	2.3	2.7	5.0

produced per primer pair ranged from 76 to 153 (Table 2). When we compared observed F_{ST} values to simulated F_{ST} values for polymorphic loci in *S. airoides* separately for each location, 45 loci (8.7%) fell above the upper 95% quantile indicating selection (Table 3). The proportion of S_L loci varied by location and by primer pair.

Forty-eight loci (9.4%) exhibited parallel trends in divergence, 42 among NS_L and 6 among S_L loci. At Greybull, Laramie, and Naturita, the proportions of greatest band presence in the invaded community were 0.372, 0.359, and 0.353 (product = 0.047). The proportions of greatest band presence in the noninvaded communities were 0.320, 0.329, and 0.269 (product = 0.027). The proportion of loci expected to exhibit parallel trends in divergence, therefore was calculated as the sum of the products for each community type (0.047 + 0.027 = 0.074, or 7.4%). We observed no excess of parallel trends in NS_L loci ($\chi^2 = 1.67$, $P = 0.196$) or in S_L loci ($\chi^2 = 2.25$, $P = 0.133$), although there were nearly two times as many observed S_L loci showing parallel trends than expected (Table 5).

Differences in genetic similarities within a community and between communities at each location showed a different

pattern than *H. comata*. That is, when using S_L loci, the similarity between communities was smaller (0.617) than the similarity within communities (0.650) whereas the two measures were similar (between = 0.676, within = 0.679) when using NS_L loci (Table 4).

Discussion

In both native grass species, small portions (*Hesperostipa comata*: 2.6%, *Sporobolus airoides*: 8.7%) of scored loci are either potentially under selection or linked to genes under selection. Those S_L loci show great differentiation while the majority of loci show relatively weak differentiation. Our proportions of S_L loci are consistent with other findings using similar methods. Wilding *et al.* (2001) identified 5% of sampled AFLP markers of the snail *Littorina saxatilis* which may have been linked to portions of the genome under selection, whereas Campbell & Bernatchez (2004) found approximately 3% of the sampled lake whitefish (*Coregonus clupeaformis*) genome associated with selection. It is unfortunate that there are not more plant species examined with these methods to allow for comparison of

Table 4 Average similarity coefficients within (diagonal) and between (off-diagonal) communities invaded by *Acroptilon repens* (I) and non-invaded communities (O) at each sampling location for *Hesperostipa comata* and *Sporobolus airoides*. Similarity values were calculated for SL loci (below diagonal, italics) and NSL loci (above diagonal, normal). Bold values denote comparisons within communities. Approximate *p*-values from 1000 random permutations of the respective individuals (Leonard *et al.* 1999) are shown in parentheses

<i>H. comata</i>	<i>n</i>	MLI	MLO	LAI	LAO	RVI	RVO	Invaded	Noninvaded
MLI	29	0.897	0.726						
		0.657	(< 0.01)						
MLO	28	<i>0.661</i>	0.813						
		(< 0.01)	0.782						
LAI	13			0.781	0.802				
				0.571	(0.83)				
LAO	15			<i>0.638</i>	0.818				
				(0.11)	0.753				
RVI	27					0.806	0.804		
						0.836	(0.40)		
RVO	27					<i>0.754</i>	0.801		
						(< 0.01)	0.752		
Invaded								0.828	0.777
								0.688	
Noninvaded								<i>0.684</i>	0.811
									<i>0.762</i>

<i>S. airoides</i>	<i>n</i>	NAI	NAO	GBI	GBO	LAI	LAO	Invaded	Noninvaded
NAI	29	0.681	0.677						
		0.624	(0.08)						
NAO	28	<i>0.573</i>	0.679						
		(< 0.01)	0.616						
GBI	26			0.687	0.689				
				0.632	(0.41)				
GBO	24			<i>0.621</i>	0.690				
				(< 0.01)	0.696				
LAI	29					0.674	0.668		
						0.668	(0.69)		
LAO	26					<i>0.657</i>	0.663		
						(0.03)	0.665		
Invaded								0.681	0.676
								0.641	
Noninvaded								<i>0.617</i>	0.677
									0.659

GB, Greybull; LA, Laramie; ML, Mud Lake; NA, Naturita; RV, Riverton; *n*, number of individuals.

our results to other studies. It must be noted that with such methodology, outliers are expected simply by chance, and results must be interpreted with caution. Before S_L loci identified in this study can be confidently regarded as under the effect of directional selection, concordant results from an independent source (i.e. quantitative trait loci mapping) should be considered (Campbell & Bernatchez 2004). Additionally, field and greenhouse studies to evaluate the relative performance of our native grass populations would further elucidate phenotypic differences, and their potential to tolerate *Acroptilon repens* competition.

The proportion of loci designated as S_L is perhaps smaller than we would have expected from self-fertilizing

plants. However, the high levels of polymorphism observed are not expected for principally self-pollinating species (Ferdinandez *et al.* 2005). Our reports of mating systems were based on a historical study (Fryxell 1957), with which other research has also shown a discrepancy (Ferdinandez *et al.* 2005). Levels of polymorphism in these two grasses may reflect a higher level of outcrossing than originally suspected, but this explanation would have to be further assessed.

The observed patterns of parallel divergence of S_L and NS_L loci seem to be consistent with an expectation that parallel selection is occurring on S_L across locations. Overall, few loci exhibited parallel trends in divergence for either grass species. Our observations of parallel divergence

Table 5 Chi-squared (χ^2) analysis of parallel trends in divergence in two grasses. Tests compare observed number of parallel trends to the number expected under neutrality for NS_L and S_L in two community types: invaded by *Acroptilon repens*, noninvaded

	Observed	Expected	χ^2 value	P value
<i>Hesperostipa comata</i>				
NS _L Parallel trend	22	20.1	0.2	
NS _L No parallel trend	352	353.9	0.01	0.664
S _L Parallel trend	2	0.5	4.0	
S _L No parallel trend	8	9.5	0.2	0.040
<i>Sporobolus airoides</i>				
NS _L Parallel trend	42	34.7	1.6	
NS _L No parallel trend	423	430.3	0.1	0.196
S _L Parallel trend	6	3.4	2.1	
S _L No parallel trend	39	41.6	0.2	0.133

are comparable to observations in lake whitefish (*Coregonus clupeaformis*) which occur in two distinct morphotypes (dwarf, normal) where around 12% (Campbell & Bernatchez 2004) of polymorphic loci (we observed 8% and 10%) showed parallel trends. Despite the many differences between fish and perennial grasses, it is interesting to note the similarity of the observations. Our grasses have only been subject to altered environmental conditions for a short time, but exhibit nearly as many parallel trends as the whitefish. That our grasses may be capable of self-fertilization might contribute to the rapidity of selection, as differentiation is highly likely in taxa with high levels of self-fertilization (Linhart & Grant 1996). Currently, there are no easily observable morphological differences between these grasses in the different communities. Because some are present in knapweed invasions while others are in areas free of knapweed, there may be physiological differences that allow persistence and require further study.

By comparing genetic similarity within and between community types, we assessed whether observed divergence patterns could be attributed to crossing of community boundaries. Individuals were more similar to one another within community types (vs. between community types) leading us to tentatively conclude that alteration of site characteristics by *A. repens* invasion may be capable of driving divergence in native grass populations.

Divergence in the face of significant amounts of gene flow is possible if selective pressure is great enough (Antonovics & Bradshaw 1970; Al-Hiyaly *et al.* 1988). We sampled mature adult plant material instead of seed. By doing so, we have described those individuals present in old exotic invasions but may not represent new recruitment into the sampled communities. Additionally, we must assume that the non-invaded communities we sampled represent pre-invasion pools of genetic information because we were not present

to sample these communities prior to invasion. A more direct test of our hypothesis would be to sample pre- and post-invasion communities, but logistic restraints (a 70-year waiting period) would prevent such a study. The invasion and dominance of a community by *A. repens* is a rapid event, in evolutionary terms. Thirty-five to 70 years is a short time for a grass population to have diverged into two genetically distinct populations even when converted to an invasive-plant dominated community.

Our results do not provide unequivocal evidence that invasive species dominance induces selection in native grass populations, but that there may be potential for localized selection between these two communities. We saw little evidence of new recruitment of our grasses in either community, and it may be a reasonable assumption that the plants we sampled have persisted in the invaded community since before the introduction of *A. repens*. If our study simply documents differences attributable to stochastic forces associated with the loss of individuals in invaded populations, it is unusual that similar patterns would occur in two different grass species at multiple locations across the western USA. *H. comata* and *S. airoides* demonstrate a presence of loci potentially linked to selection between the two community types; loci that also exhibit a high degree of parallel divergence. Greater similarity within a community than between communities suggests that differences are not tied solely to physical distance on the landscape. If natives in invaded communities have simply persisted since before invasion, they may have persisted for a reason, not simply by chance (see Callaway *et al.* 2005). Without morphological and plant performance data to provide a measure of trait differences between community types, we are reluctant to relate perceived differentiation to increased tolerance of conditions within *A. repens* invasions, although, given our results and the common use of these species in restoration, such study is warranted.

Huff *et al.* (1998) asserted that 'a native grass population is more than just a Latin binomial. Evolutionary forces often create something unique and irreplaceable at the local level.' Evolutionary forces should act upon native species once a new environment has been constructed around them in the same way that invasives adapt to new environments. We suggest that plant invasions may affect the evolutionary trajectories of native plant populations in ways not previously investigated. More extensive research of native population response to invasions is needed to successfully address potential genetic changes caused by exotic invasion.

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