Rangeland Ecology & Management 71 (2018) 443–448

Contents lists available at ScienceDirect

Rangeland Ecology & Management

journal homepage: http://www.elsevier.com/locate/rama

Original Research

Trait Response and Change in Genetic Variation upon Selection for Spike Number in Salina Wildrye

T.A. Jones *, S.R. Larson

US Department of Agriculture —Agricultural Research Service, Forage and Range Research Laboratory, Logan, UT 84322-6300, USA

ARTICLE INFO

Article history:
Received 20 November 2017
Received in revised form 20 February 2018
Accepted 14 March 2018

Key Words:
genetic bottleneck
genetic similarity
inbreeding depression
recurrent selection
seed yield

ABSTRACT

Salina wildrye (*Leymus salinus* [M.E. Jones] Á. Löve) is a perennial cool-season grass that potentially could become an important restoration species in the Colorado Plateau. However, its seed production has never been commercially viable due to sparse heading. We compared a 4*x* ssp. *salmonis* population, Lakeside C_3, to an 8*x* ssp. *salinus* population, 9043501, for seed production—related traits and measured the response of 9043501 to 2 cycles of selection for increased spike number over a 4-yr period at Millville, Utah. Seed yield of Lakeside and 9043501 was similar ($P > 0.10$) in 2013, but seed yield of 9043501 was 81% greater ($P < 0.10$) than Lakeside in 2014 and 191% greater ($P < 0.01$) in 2015. Lakeside spike number was 99% greater ($P < 0.0001$) than 9043501 in 2013, but they were similar ($P > 0.10$) in 2014 and 2015. Seeds per spike of 9043501 were 71% ($P < 0.05$), 80% ($P < 0.05$), and 209% ($P < 0.01$) greater than Lakeside in 2013, 2014, and 2015, respectively. Selection in 9043501 increased ($P < 0.05$) spike number by 4.3 spikes per plant (19.8%) per cycle of selection in the first seed-production yr (2013), but no change was seen in 2014 or 2015 ($P > 0.10$). Selection did not change ($P > 0.10$) seeds per spike or individual seed mass. Consequently, seed yield increased ($P < 0.05$) 0.32 g per plant per cycle (36.8%) in 2013, with no increase ($P > 0.10$) in 2014 or 2015. Dry matter per plant across the 4 yr increased ($P < 0.01$) 10.3 g per plant per cycle (9.3%), and canopy height increased ($P < 0.01$) 3.9 cm per cycle (6.6%) in 2013. AFLP DNA primers detected a 1.7% loss of genetic variation per cycle, presumably due to a combination of selection and genetic drift, but no plant traits were diminished as a result.

Introduction

Salina wildrye (*Leymus salinus* [M.E. Jones] Á. Löve = *Elymus salinus* M. E. Jones) is a moderately rhizomatous, cool-season perennial grass native to the Intermountain West (Monsen et al., 2004, p. 381—382). This grass could be used for restoration of harsh, arid, disturbed sites such as those that have been developed for oil or gas production. It may grow on a variety of soil textures ranging from rocky to loams and clays, occupying hillsides, alluvial fans, plateaus, bluffs, canyons, and montane areas as part of salt desert shrubland, sagebrush-grass, mountain mahogany, aspen, and conifer plant communities (Baker and Kennedy, 1985; Vallentine, 1989; Monsen et al., 2004, p. 381—382). *Leymus salinus* ssp. *salinus* is prevalent east of the Wasatch Mountains in eastern Utah, western Colorado, and southwestern Wyoming (Barkworth and Atkins, 1984, Fig. 6) and may be found in association with the saltbush shrub species *Atriplex confertifolia* and *A. gardneri* (Baker and Kennedy, 1985). A second subspecies of *L. salinus,* ssp. *salmonis,* occurs west of the Wasatch Mountains in eastern Idaho, western Utah, and Nevada. While ssp. *salinus* is relatively frequent within its range, the distribution of ssp. *salmonis* is much spottier (Atkins et al., 1984). As far as is known, ssp. *salmonis* is tetraploid ($2n = 28$), while ssp. *salinus* may be tetraploid, hexaploid ($2n = 42$), or octoploid ($2n = 56$) (Atkins et al., 1984). A third subspecies, ssp. *mojavensis,* occurs in southern California and Arizona (Barkworth and Atkins, 1984). *Leymus salinus* and *L. cinereus* have been shown to be the parents of *L. ambiguus* (Culumber et al., 2011, Fig. 2), which occurs on the eastern slopes of the Rocky Mountains in either tetraploid or octoploid states (Atkins et al., 1984).

Because no plant materials of salina wildrye have been released for commercial seed production, only wildland-collected seed has been available for seeding operations, which means that seed quality is often poor, seed prices are high, and seed availability is limited. However, 9043501, an octoploid population collected in northeastern New Mexico (Colfax County), has been identified by the Upper Colorado Environmental Plant Center in Meeker, Colorado as a promising plant material (Monsen et al., 2004). This population keys to *L. salinus* ssp. *salinus* because it possesses neither the pubescent basal leaves of ssp. *salmonis* nor the flat leaf blades of ssp. *mojavensis* (Barkworth and Atkins, 1984); that is, its leaves are glabrous and strongly involute.

* Correspondence: Thomas A. Jones, Forage & Range Research Laboratory, USDA-ARS, 696 N 1100 E, Logan, UT, 84322-6300, USA.
E-mail address: thomas.jones@ars.usda.gov (T.A. Jones).

https://doi.org/10.1016/j.rama.2018.03.005
1550-7424/

The greatest limiting factor to the adoption of this species in the seed trade is its poor seedling establishment and low seed yield relative to other cool-season perennial rangeland grasses. Low seed yield is primarily due to the production of relatively few inflorescences (spikes) per plant. For this reason, we conducted two cycles of phenotypic recurrent selection for increased spike number in an attempt to increase seed production potential of 9043501. As *L. salinus* is an allogamous species, hybridization occurs between plants each generation, forcing genetic recombination and making it a logical candidate for recurrent selection. Before this selection, we had conducted two cycles of selection for salinity tolerance, but response to that earlier selection is not germane to this study. In an evaluation of response to selection at Millville, Utah, we included the octoploid 9043501, the four cycles (populations) of selection on 9043501, and Lakeside C_3, a tetraploid ssp. *salmonis* population used for comparison with 9043501.

We had three objectives in this study. Objective 1 was to compare the *L. salinus* ssp. *salmonis* Lakeside C_3 population ($2n = 4x = 28$) to the *L. salinus* ssp. *salinus* 9043501 C_0 population ($2n = 8x = 56$) (henceforth 9043501) for the previously mentioned traits. Objective 2a was to evaluate the effect of two cycles of selection on 9043501 for spike number per plant (C_3, C_4) on spike number per plant, seed yield per plant, and the two other components of seed yield, seeds per spike and individual seed mass. Objective 2b was to evaluate the effects of selection on 9043501 on two vegetative traits, dry matter per plant and canopy height, and two germination traits, percentage and rate. Objective 3 was to measure genetic similarity within 9043501 and each of four cycles of selection in 9043501, two (C_1, C_2) for salinity tolerance and two (C_3, C_4) for spike number. This was done to determine whether genetic variation had been lost from the 9043501 base population as a consequence of artificial selection. Genetic variation can be lost from populations subjected to selection primarily due to genetic drift, a process by which a population is genetically narrowed due to a relatively small number of individuals, leading to unavoidable mating of relatives and consequential inbreeding.

Materials and Methods

Selection History

Four cycles of selection have been practiced on 9043501. Selection was applied for tolerance to salinity in the first two cycles and for spike number (ocular estimate) in the latter two cycles. For C_1, seeds were germinated in an EC = 24 solution in the laboratory (Peel et al., 2004). Seeds that germinated were planted individually into 1 020 silica sand-filled cones in a greenhouse and subjected to a salinity protocol (dunked in saline solution 2× per week), escalating from EC = 12 (17 February 1997) to EC = 48 (31 March 1997). In April 1997, 10% of the plants were selected for vigor under these conditions (i.e., salinity tolerance, and transplanted into fresh containers). These selections were transplanted to Greenville Farm (Cache County, Utah) on 30 May 1997, and recombined seed was harvested in 1998 (C_1). For C_2, C_1 seeds were germinated in an EC = 33 solution. Once the seedlings recovered from this stress, they were subjected to a salinity protocol escalating from EC = 6 (1 February 1999) to EC = 42 (11 April 1999). In April 1999, 10% of the plants (1 320 individuals) were selected for salinity tolerance and transplanted into fresh containers before transplanting to North Park Farm (Cache County, Utah) in May. Recombined seed was harvested in 2000 (C_2).

In January 2006, 686 C_2 seedlings were transplanted to greenhouse flats, and on 21 March 2006 38.0% were selected for high seedling vigor based on high tiller number and size. On 14 April 2006, a 252-plant nursery was established on 0.40-m centers at Blue Creek Farm (Box Elder County, Utah). On 18 May 2007, 40 plants (15.9%) were selected because each displayed a greater number of spikes than its eight nearest neighbors. Spikes were removed from unselected plants, and the selections were intermated to produce seed in 2007 (C_3). For C_4, 980 C_3 seedlings were transplanted to greenhouse flats in January 2008, and on 26 March 2008, 358 seedlings were selected for seedling vigor as described earlier. On 2 May 2008, 336 of these selections were transplanted to Blue Creek Farm on 0.30-m centers. On 16 June 2009, 42 plants (12.5%) were selected for spike number relative to the eight nearest neighbors, as described earlier, but spikes were not removed from unselected plants as in 2007 for the previous cycle. Open-pollinated seed was harvested from these 42 plants later that summer (C_4).

Lakeside C_3 (henceforth Lakeside), the tetraploid check, resulted from three cycles of selection, each of which included selection for vigor score, seed yield, and emergence from deep seeding, on the Lakeside C_0 population. Lakeside C_0 is a tetraploid ssp. *salmonis* population generated by bulking collections at > 20 sites in the Stansbury Mountains southwest of the Great Salt Lake in Tooele County, Utah.

Millville Evaluation Trial

A replicated evaluation trial comparing 9043501, four cycles of selection from 9043501, and Lakeside was established at Millville Farm (Cache County, Utah) 16 May 2012. The six populations were planted in 10-plant plots in a 5 × 2 arrangement on 30-cm centers with a 60-cm alleyway between plots in both directions. Plots were arranged in a randomized complete block design with 8 replications. Dry matter production was measured 27 September 2012 (5-cm height), 18 July 2013 (10 cm), 16 September 2014 (10 cm), and 5 August 2015 (10 cm). Canopy height was measured 6 June 2013, but not in the following years. Spikes were counted 6 June 2013, 13 June 2014, and 15 June 2015. Seed was harvested before shattering commenced, and seed mass was determined from four 100-seed samples per plot in all 3 seed-production yr.

Germination percentage and rate (Maguire, 1962) were measured on seed harvested in 2013 and 2014. Germination boxes were lined with blotter paper (Anchor Paper Company, Plymouth, MN), with each containing 100 seeds dusted with thiram fungicide. Four boxes were prepared for each plot in the field design, and water was added to begin the trials 29 August 2014 for 2013-harvested seed and 27 November and 4 December 2015 (four field replications each) for 2014-harvested seed. Tests were conducted at 22°C. Germinated seedlings were tallied daily from d 5 (following watering), the onset of germination, through d 24 (when germination was essentially complete), except for boxes for which counts were terminated earlier because 3 successive d without germination were recorded.

DNA Variation

Young leaf tissues from 192 seedlings, including at least 32 individuals from each cycle (population) of selection, 9043501 and $C_1 - C_4$, were lyophilized and milled using a MM300 (Retsch Inc., Newtown, PA) mixer mill for DNA extraction using the DNeasy 96 Plant Kit (Qiagen, Germantown, MD). We obtained complete DNA profiles (six AFLP primer pairs) for 171 seedlings, including at least 27 individuals from each population, using the AFLP technique (Vos et al., 1995) with modifications for detection using fluorescent labels and capillary electrophoresis. Briefly, the selective *Eco*RI primers were fluorescent labeled with 6-FAM and fractionated by capillary electrophoresis on an ABI3100 instrument (PE Applied Biosystems, Foster City, CA) with internal GS-500 size standards (PE Applied Biosystems) for each sample in each channel. The *Eco*RI and *Mse*I preamplification primers both included a single selective nucleotide, A and C, respectively. The selective amplification primers included two additional selective nucleotides to make six different primer combinations: E.AAG//M.CAA, E.ACA//M.CCC, E.ACA//M.CGG, EACG//M.CAA, E.ACG//M.CTC, and E.ACT//M.CTC. Different AFLP markers were identified and scored for the presence or absence of bands (DNA amplicons) on the basis of the relative mobility corresponding to about 1 bp using Genographer open-source software.

Genetic diversity of each cycle was compared on the basis of the average proportion of shared amplicons (S) among individual plants using the formula $2N_m/(N_x + N_y)$, where N_m is the number of shared amplicons and N_x and N_y are the numbers of amplicons in plants X and Y. Thus, the average proportion of shared amplicons can range from zero (no shared bands) to 1 (identical profiles), as an inverse function of genetic diversity, but the absence of bands in both plants does not count as a shared character. Variances and standard errors for the average genetic similarity (S) within each cycle were used for comparing levels of genetic diversity using *t*-tests (Leonard et al., 1999).

Results

Comparison of Lakeside and 9043501

Performance of Lakeside was similar to that of 9043501 early in the trial, but as time progressed differences became more conspicuous, with the notable exceptions of spike number per plant and seed mass (Table 1). Spike number in 2013 was 49.7% lower ($P < 0.05$) for C_0 than Lakeside, while in 2014 and 2015 spike numbers of 9043501 and Lakeside were similar ($P > 0.10$). Nevertheless, Lakeside's higher 2013 spike number did not translate into a seed yield advantage ($P > 0.10$) in that year. In 2014, seed yield of 9043501 exceeded Lakeside by 81% ($P < 0.10$) and again by 191% ($P < 0.01$) in 2015. This was primarily due to increasing numbers of seeds per spike for 9043501 relative to Lakeside over time, namely 71% ($P < 0.05$) in 2013, 80% ($P < 0.05$) in 2014, and 209% ($P < 0.01$) in 2015. Summed over the 3 yr, seed yield of 9043501 exceeded Lakeside by 60% (3.23 vs. 2.02 g/plant) ($P < 0.05$). In contrast, seed mass of Lakeside and 9043501 was similar ($P > 0.10$) in 2013 and 2015, but in 2014 Lakeside exceeded 9043501 by 7.8% ($P < 0.05$).

The two populations were similar ($P > 0.10$) for dry matter in the 2012 establishment year and again in 2013 ($P > 0.10$). However, as the plants grew older, 9043501 exceeded Lakeside in dry matter by 37.6% in 2014 ($P < 0.01$) and 34.3% in 2015 ($P < 0.0001$). Germination rate index of the two populations was similar ($P > 0.10$) in 2013, but in 2014 the index was greater for 9043501 (16.5) than for Lakeside (13.9) ($P < 0.01$). Germination percentage was greater for 9043501 than for Lakeside, both in 2013 (89.1 vs. 82.9%; $P < 0.0001$) and 2014 (93.2 vs. 82.4%; $P < 0.05$). Canopy height, measured only in the first seed-production yr (2013), was similar ($P > 0.10$) between the two populations.

Repeated Measures Analysis Over Years

Spike numbers in 2013 and 2015 were similar ($P > 0.10$), but they were lower in 2014 ($P < 0.05$) (Fig. 1a). Despite this, seed yield did not differ among years ($P > 0.10$) (Figure 1b). Overall, Lakeside exceeded 9043501 populations for spike number per plant (23.1 vs. 19.1; $P < 0.05$), but 9043501 populations exceeded Lakeside for seed mass per spike (65.6 vs. 31.8 mg; $P < 0.0001$) and overall seed yield (1.19 vs. 0.68 mg/plant; $P < 0.01$). Aboveground dry matter increased steadily from 2012 to 2015 as plants matured ($P < 0.0001$) (Fig. 1c). Dry matter production was significantly ($P < 0.05$) lower for Lakeside than for 9043501 populations except C_2. The ploidy × year interaction for dry matter was highly significant ($P < 0.0001$). While Lakeside did not differ ($P > 0.10$) from the mean of the 9043501 populations for dry matter in 2012 or 2013, the 9043501 populations displayed dramatically greater ($P < 0.0001$) dry matter in 2014 (37.9 vs. 27.5 g/plant; $F = 23.0$) and 2015 (58.7 vs. 43.4 g/plant; $F = 38.2$).

Responses to Selection

We quantified direct and indirect responses to selection from C_2 to C_4 (see Table 1). Spike number, as measured in 2013, increased an average of 4.3 spikes per plant (19.8%) per cycle ($P < 0.05$), but no response to selection ($P > 0.10$) was found for spike number in 2014 or 2015 (see Fig. 1a). Summed over 3 yr, spike number responded to selection ($P < 0.05$) at the rate of 8.3 spikes per plant (14.0%) per cycle. Response to selection was also greatest for seed yield in 2013, with seed yield increasing 0.32 g per plant (36.8%) per cycle ($P < 0.05$) (Fig. 1b). No response ($P > 0.10$) was detected for 2014 or 2015 seed yield. Neither seeds per

Table 1
Comparison of Lakeside C_3 (4*x*) and 9043501 C_0 (8*x*) salina wildrye populations and response of 9043501 to two cycles (C_2 to C_4) of selection (for spike number) for spike number per plant (2013−2015), seed yield per plant (2013−2015), seeds per spike (2013−2015), seed mass (2013−2015), dry matter per plant (2013−2015), canopy height (2013), germination percentage (2013−2014), and germination rate index (2013−2014).

Yr	Trait	Lakeside vs. 9043501 C_0			Selection of 9043501 C_2		
		C_3 Lakeside	C_0 9043501	Sig. level	Selection response per cycle	Sig. level	% change per cycle
2013	Spike number per plant	36.0	18.1	***	4.3	*	+19.8
2014	Spike number per plant	13.0	13.8	Ns	0.8	Ns	+5.4
2015	Spike number per plant	26.0	23.7	Ns	2.5	Ns	+11.5
Total	Spike number per plant	76.3	56.6	*	8.3	*	+14.0
2013	Seed yield per plant (g)	0.82	0.68	Ns	0.32	*	+36.8
2014	Seed yield per plant (g)	0.62	1.12	+	0.15	Ns	+13.9
2015	Seed yield per plant (g)	0.45	1.31	**	−0.04	Ns	−3.0
Total	Seed yield per plant (g)	2.02	3.23	*	0.68	Ns	+19.3
2013	Seeds per spike	6.2	10.6	*	1.1	Ns	+8.5
2014	Seeds per spike	12.2	21.9	*	1.9	Ns	+9.3
2015	Seeds per spike	5.8	17.9	**	−2.4	Ns	−11.5
2013	Seed mass (mg)	3.81	3.57	Ns	0.02	Ns	+0.5
2014	Seed mass (mg)	4.30	3.99	*	0.07	Ns	+1.9
2015	Seed mass (mg)	3.26	3.42	Ns	−0.03	Ns	−0.9
2012	Dry matter per plant (g)	4.4	4.4	Ns	0.6	Ns	+13.3
2013	Dry matter per plant (g)	18.8	17.8	Ns	3.2	Ns	+18.0
2014	Dry matter per plant (g)	27.7	38.1	**	2.3	Ns	+6.8
2015	Dry matter per plant (g)	43.4	58.3	***	2.6	**	+4.9
Total	Dry matter per plant (g)	94.3	114.2	*	10.3	**	+9.3
2013	Canopy height (cm)	58.1	59.4	Ns	3.9	**	+6.7
2013	Germination percentage	82.9	89.1	+	1.6	Ns	+1.8
2014	Germination percentage	82.4	93.2	***	1.5	*	+1.6
2013	Germination rate index	13.0	13.6	Ns	0.4	Ns	+2.6
2014	Germination rate index	13.6	16.5	**	0.4	Ns	+2.5

+, *, **, ***, Ns, Significantly different at $P < 0.10$, 0.05, 0.01, and 0.0001 and not significantly different at $P > 0.10$, respectively.

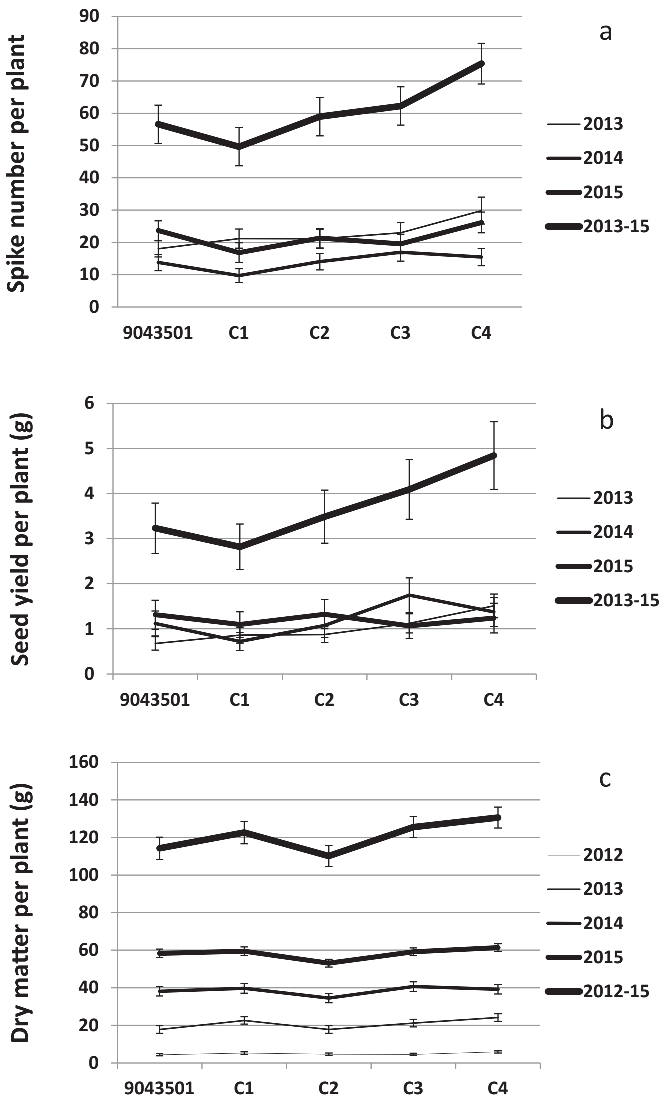

Figure 1. Response to four cycles of recurrent selection in the octoploid 9043501 population of salina wildrye for **a,** spike number per plant, **b,** seed yield per plant (g), and **c,** dry matter production per plant (g). Least-squares means and standard errors are presented.

spike nor seed mass changed ($P > 0.10$) in response to selection for spike number in any of the 3 yr.

Dry matter production did not change ($P > 0.10$) with cycles of selection except when plants were most mature (2015), when it increased ($P < 0.01$) 2.6 g per plant (4.9%) per cycle (Fig. 1c). Canopy height increased ($P < 0.01$) 3.9 cm (6.7%) per cycle in 2013, the only year in which this trait was measured. Germination percentage increased ($P < 0.05$) 1.5% per cycle in 2014, but no change ($P > 0.10$) was seen for germination percentage in 2013 or for germination rate index in either year.

Genetic Variation

A total of 363 polymorphic AFLP markers (i.e., markers with bands present and absent among the 171 plants genotyped) were scored. The average (± standard error) number of bands per plant ranged from 120.8 (± 1.6) in C_4 to 126.1 (± 1.1) in C_1. Although the average number of bands per plant was different ($P < 0.05$) between C_4 and C_1, there were no other differences ($P > 0.10$) in average number of bands per plant among the five populations, 9043501 to C_4. The average similarity indices were 0.509 (± 0.008), 0.519 (± 0.007), 0.528 (± 0.009), 0.520 (± 0.007), and 0.542 (± 0.007) for 9043501, C_1, C_2, C_3, and C_4, respectively. The average similarity coefficients for C_2, C_3, and C_4 were all greater ($P < 0.05$) than for 9043501. If genetic variation declines due to selection, genetic similarity among individuals of the selected population will increase. The genetic similarity coefficient, which may range from 0 to 1, increased from 0.5088 to 0.5419 with four cycles of selection (Fig. 2). A regression line fit to these data was significant ($P < 0.10$), indicating an increase in genetic similarity, or equivalently a loss of genetic variation.

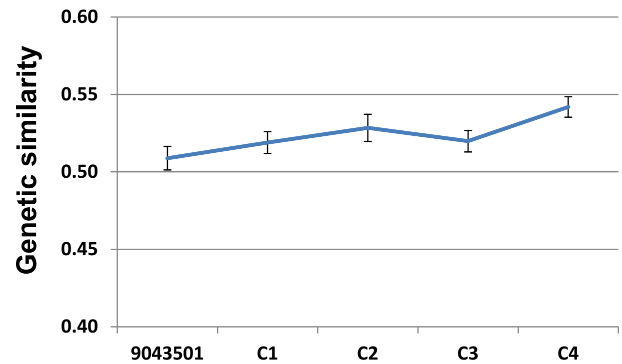

Figure 2. Changes in genetic similarity through four cycles of recurrent selection in the octoploid 9043501 population of salina wildrye. Least-squares means and standard errors are presented. Genetic similarity is the inverse of genetic variation.

Discussion

Lakeside versus 9043501 Comparison

Objective 1 was to compare Lakeside with 9043501. In 2013, the first seed-production yr, the two populations were similar ($P > 0.10$) for seed yield (see Table 1). This was because, despite double the number of spikes for Lakeside relative to 9043501 ($P < 0.0001$), the latter's greater number of seeds per spike ($P < 0.05$) compensated for its lower spike number (see Table 1). Beginning in 2014, however, relative seed yield of the two populations began to diverge ($P < 0.10$) in favor of 9043501, with that divergence increasing ($P < 0.01$) in 2015. This was not only because 9043501 spike numbers increased from 2013 to become similar ($P > 0.10$) to Lakeside in 2014 and 2015, but also because 9043501 seeds per spike exceeded Lakeside by 80% in 2014 ($P < 0.05$) and by 204% in 2015 ($P < 0.01$). At the same time, differences in seed mass were relatively minor, with Lakeside having an 8% advantage over 9043501 in 2014 ($P < 0.05$) but with no differences seen in 2013 or 2015 ($P > 0.10$). Germination percentage and germination rate index also favored 9043501, but this difference was much more pronounced in 2014 ($P < 0.0001$) than in 2013 ($P < 0.10$) (not measured in 2015). Dry matter production steadily increased over years for both populations (Fig. 1c), but interestingly this annual increase in biomass was not associated with increasing spike number (Fig. 1a). Whereas these two populations were similar ($P > 0.10$) for dry matter production in 2012 and 2013, 9043501 exceeded Lakeside by 38% in 2014 ($P < 0.01$) and by 34% in 2015 ($P < 0.0001$) (see Table 1). Thus, over time 9043501 was increasingly favored over Lakeside for dry matter, seed yield, spike number, seeds per spike, germination percentage, and germination rate index, while no trend was seen for seed mass.

Plant Response to Selection

Objective 2a was to assess the impact of selection of 9043501 on seed yield and its components. Two cycles of selection increased ($P < 0.05$) spike number 29.2% per cycle in the first seed-production yr (2013), which accompanied a 36.8% increase in seed yield ($P < 0.05$) per cycle in that yr (see Table 1). Selection for spike number was conducted in the first seed-production yr, which may explain why a response was detected in 2013 ($P < 0.05$) but not in later years ($P > 0.10$) (see Table 1). Selection for spike number did not change ($P > 0.10$) seeds per spike or seed mass in any of the 3 yr, suggesting the absence of a genetic correlation between spike number and these other seed-yield components.

Spike number could have increased due to the removal of morphological or physiological constraints to reproductive tillering (Mueller and Richards, 1986) or due to an ontogenic effect that hastened reproductive maturity. However, these two mechanisms are not mutually exclusive, as the removal of a tillering constraint could serve as a mechanism for an ontogenic shift (shorter juvenile period). The fact that selection increased spike number in 2013 ($P < 0.05$) but not in 2014 or 2015 ($P > 0.10$) suggests an ontogenic response. We did not detect a change in plant longevity associated with such a response, as no plant mortality was seen beyond the establishment yr (2012).

An ontogenic shift may be desirable in a restoration setting. Early-seral grasses and forbs establish better on Great Basin rangelands than late-seral species but also survive and compete better against medusahead wildrye (*Taeniatherum caput-medusae* [L.] Nevski) (Uselman et al., 2014, 2015). Indigenous populations of native perennial grasses display genetic shifts toward earlier phenology (Kulpa and Leger, 2013) and greater competitive ability subsequent to invasion by cheatgrass (*Bromus tectorum* L.) (Goergen et al., 2011).

Objective 2b was to determine whether selection for spike number had effects on vegetative and germination traits. Selection did not increase ($P > 0.10$) dry matter per plant in 2012 − 2014, but in 2015, when dry matter was highest, it did increase ($P < 0.01$) in response to selection. Totaled across the 4 yr, dry matter increased ($P < 0.01$) 9.3% per cycle (see Table 1, Fig. 1c). Selection increased ($P < 0.01$) canopy height 3.9 cm (6.7%) per cycle in 2013, the only year that it was measured. Thus, both of our vegetative dependent variables, dry matter and canopy height, increased in response to selection for spike number. We did not measure root biomass, so we cannot state whether a negative genetic correlation between aboveground and belowground dry matter production might have occurred. Though no response ($P > 0.10$) was seen in 2013, in 2014 germination percentage increased ($P < 0.05$) 1.5% per cycle in response to selection. Germination rate index, on the other hand, did not change ($P > 0.10$) in either 2013 or 2014. Thus, selection appears to have had only a modest effect on germination.

Loss of Genetic Variation Due to Selection

Objective 3 was to determine whether genetic variation was lost as a consequence of selection. Using the values presented at the end of the *Results* section, we found a genetic loss over the course of four cycles of selection of $(0.5419 - 0.5088)/(1.0 - 0.5088) = 6.7\%$ ($P < 0.10$) of the genetic variation present in C_0 (i.e., a 1.7% loss per cycle). Genetic loss may result from 1) fixation or near-fixation of selected DNA variants (alleles), 2) 'hitchhiked' alleles that are inadvertently selected due to their proximal position to selected loci (termed "selective sweep"), or 3) a small population size in a cycle of selection (termed "genetic drift" or "genetic bottleneck"). The observed genetic loss, then, is a confounded result of selection and demographic effects (genetic drift). Although AFLP markers are generally considered to be selectively neutral (i.e., related to noncoding regions of DNA rather than coding genes that are subject to selection), they encompass the entire genome, both coding and noncoding regions. Over an estimated 2 800 cycles of selection, modern maize (*Zea mays* ssp. *mays*) was domesticated from teosinte (*Zea mays* ssp. *parviglumis*) through selection at an estimated 2 − 4% of the total number of genes (Wright et al., 2005). Thus, it can be seen that selection, even over a long extent, involves only a small portion of the total number of coding genes. However, though most AFLP markers are probably not located in genes, they could be affected by linkage to genes under selection. Overall, selection is likely responsible for only a minor portion of the loss of genetic variation, as measured by loss of AFLP variation, with the lion's share instead being due to demographic effects.

As salina wildrye is a cross-pollinating species, presumably it is more susceptible to inbreeding depression than would be expected for a self-pollinating species (Husband and Schemske, 1996). Did the loss of genetic variation negatively impact measured traits? If this were an issue, one might expect a decline in some of the 21 trait × year combinations we measured (see Table 1). Of these, we saw increases ($P < 0.05$) for 5 and no change ($P > 0.10$) for 16, but no declines ($P < 0.10$).

The goal of recurrent selection is to improve the base population for the trait(s) under selection by applying selection pressure (i.e., selecting only those plants that meet or exceed a threshold value while discarding all others). The more stringent the selection intensity, the fewer the selected plants as a percentage of the total number of plants evaluated. This creates a dilemma, however, because as the absolute number (not percentage) of plants selected declines, increasing selection intensity, the greater the genetic drift or bottleneck of the population. This causes a stochastic loss of genetic variation, increased inbreeding, and ultimately reduced fitness. Thus, in order for selection to increase fitness, its benefits must exceed the costs of genetic loss. The breeder aims for a selection intensity that is neither too high, leading to inbreeding, nor too low, limiting fitness increases.

Losses of genetic variation in selected populations may be detected (Chivers et al., 2016). Although some might consider a reduction of genetic variation, as found here, to be an indictment of recurrent selection, a reduction of genetic variation need not be an irreparable consequence. Corrective action may be taken by introducing new genetic variation that may compensate for that which has been lost, thus ameliorating the negative impacts. Introduction of even small numbers of unaffected individuals to a compromised population can provide dramatic relief of inbreeding depression (Swindell and Bouzat, 2006). We refer to this course of action as "detect and correct." We strongly recommend monitoring genetic variation in selected populations so that its loss can be detected and then rectified.

Practical Considerations

Questions are sometimes raised concerning the similarity of the selection or evaluation environment to the target environment in which the seed will actually be used. This is complicated by the fact that there are really two sets of target environments: 1) the arable site where the seed is increased and 2) the wildland site where the restoration project is located. Further complicating the issue, the two sets of environments are typically dissimilar. We aim to develop plant materials that perform better in both sets of environments. Any progress we have made toward increased spike number in our selection in northern Utah is only of value to the extent that it is correlated with progress in Washington seed fields or wildland sites on the Colorado Plateau. Analogous to this issue is another, namely that the selection and evaluation of our salina wildrye material has been conducted in spaced-plant nurseries rather than the seeded rows that are typically used in seed fields and restoration plantings. Even though the seeds are seeded in rows in a restoration setting, so few establish that they are akin to spaced plants. We now routinely decrease our plant spacing with advancing cycles of selection to increase the environmental challenge to the plants. For both issues, the problem is acute when the genotype-by-environment interaction is high relative to the main effect of genotype. When heritability of the trait in question is high, however, the problem is minor.

The provisioning of seed of a diversity of species and a variety of plant materials is desirable for restoration practice, but its delivery is challenging when large quantities of seed are required over extensive and diverse geographical areas (Broadhurst et al., 2016). Some species are more tractable than others in this regard. Species that display poor seed yield or present difficulties in seed harvest or conditioning, in particular, are unlikely to become available for widespread restoration use. However, the work presented here shows that research can sometimes address the problem of inherently low seed production to expand the plant-materials palette of the restoration practitioner. This is part of our ongoing work to bring recalcitrant native species to market. Previous efforts have stimulated the widespread use of Indian ricegrass (*Achnatherum hymenoides* [Roem. & Schult.] Barkworth) by the release of "Rimrock" (Jones et al., 1998), which displays a morphological feature that limits seed-shattering losses (Whalley et al., 1990; Jones and Nielson, 1992), thereby reducing the costs of seed production. "Workhorse species" status has been achieved for bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey) by developing several plant materials of this species (e.g., Toe Jam Creek Germplasm) (Jones et al., 2004), which produce reasonably large quantities of seed under cultivation.

Data presented here demonstrate that selection for increased spike number in salina wildrye has increased the number of seeds produced per plant (i.e., fecundity). Fecundity is one of two components of ecological fitness, as ecological fitness = fecundity × survivorship to reproductive maturity. Thus, all else being equal, increased fecundity increases ecological fitness. In the challenging environments where salina wildrye is found, increased ecological fitness is likely advantageous for restoration of ecosystem structure and function. However, as seen from the equation shown earlier, fecundity is only half of the equation. For maximal benefit from increased fecundity, the survivorship issue must also be addressed. Many restoration environments are greatly modified environments, and in some cases, ecological thresholds have been breached. In such environments, survivorship can be especially critical. This is why we emphasize seedling vigor and establishment in our program, in addition to fecundity.

Implications

Priming natural selection in the wild with adaptive variation from an ex situ source, such as the plant material developed here, is a strategy of "assisted evolution" (Jones and Monaco, 2009). Assisted evolution considers both empirical performance and genetic identity. Here we used the age-old plant breeding technique of artificial selection to create material that features the "genetic background" of the 9043501 C_0 base population yet displays greater fecundity. Restoration practitioners might be able to jump-start the evolutionary process of natural selection by introducing such genetic material that provides both high mean performance for the present and genetic variation for the future. As such, assisted evolution is a pragmatic approach that may provide a useful plant-materials option for restoring systems that are no longer pristine.

Acknowledgments

Thanks to Kevin Jensen and the Upper Colorado Environmental Plant Center for providing seed of the Lakeside C_3 check population and 9043501, respectively. Thanks also to two anonymous reviewers for their insightful comments.

References

Atkins, R.J., Barkworth, M.E., Dewey, D.R., 1984. A taxonomic study of *Leymus ambiguus* and *L. salinus* (Poaceae: Triticeae). Systematic Botany 9, 279–294.
Baker, W.L., Kennedy, S.C., 1985. Presettlement vegetation of part of northwestern Moffat County, Colorado, described from remnants. Great Basin Naturalist 45, 747–778.
Barkworth, M.E., Atkins, R.J., 1984. *Leymus* Hochst. (Gramineae: Triticeae) in North America: taxonomy and distribution. American Journal of Botany 71, 609–625.
Broadhurst, L., Jones, T.A., Smith, F.S., North, T., Guja, L., 2016. Managing seed resources for restoration in an uncertain future. BioScience 66, 73–79.
Chivers, I.H., Jones, T.A., Broadhurst, L.M., Larson, S.R., Mott, I.W., 2016. The merits of artificial selection for the development of restoration-ready plant materials of native perennial grasses. Restoration Ecology 24, 174–183.
Culumber, C.M., Larson, S.R., Jensen, K.B., Jones, T.A., 2011. Genetic structure of Eurasian and North American *Leymus* (Triticeae) wildryes assessed by chloroplast DNA sequences and AFLP profiles. Plant Systematics and Evolution 294, 207–225.
Goergen, E.M., Leger, E.A., Espeland, E.K., 2011. Native perennial grasses show evolutionary response to *Bromus tectorum* (cheatgrass) invasion. PLoS One 6, e18145.
Husband, B.C., Schemske, D.W., 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50, 54–70.
Jones, T.A., Monaco, T.A., 2009. A role for assisted evolution in designing native plant materials for domesticated landscapes. Frontiers in Ecology and the Environment 7, 541–547.
Jones, T.A., Nielson, D.C., 1992. High seed retention of Indian ricegrass PI 478833. Journal of Range Management 45, 72–74.
Jones, T.A., Majerus, M.E., Scheetz, J.G., Holzworth, L.K., Nielson, D.C., 1998. Registration of Rimrock Indian ricegrass. Crop Science 38, 539–540.
Jones, T.A., Nielson, D.C., Larson, S.R., Johnson, D.A., Monaco, T.A., Caicco, S.L., Ogle, D.G., Young, S.A., Carlson, J.R., 2004. Registration of Toe Jam Creek bottlebrush squirreltail germplasm. Crop Science 44, 1880–1881.
Kulpa, S.M., Leger, E.A., 2013. Strong natural selection during plant restoration favors an unexpected suite of plant traits. Evolutionary Applications 6, 510–523.
Leonard, A.C., Franson, S.E., Hertzberg, V.S., Smith, M.K., Toth, G.P., 1999. Hypothesis testing with the similarity index. Molecular Ecology 8, 2105–2114.
Maguire, J.D., 1962. Speed of germination—aid in selection and evaluation for seedling emergence and vigor. Crop Science 2, 176–177.
Monsen, S. B., Stevens, R., and Shaw, N. L. [compilers]. 2004. Restoring western ranges and wildlands. Gen. Tech. Rep. RMRS-GRT-136, vol. 2. Fort Collins, CO, USA: US Department of Agriculture, Forest Service, Rocky Mountain Research Station.
Mueller, R.J., Richards, J.H., 1986. Morphological analysis of tillering in *Agropyron spicatum* and *Agropyron desertorum*. Annals of Botany 58, 911–921.
Peel, M.D., Waldron, B.L., Jensen, K.B., Chatterton, N.J., Horton, H., Dudley, L.M., 2004. Screening for salinity tolerance in alfalfa: a repeatable method. Crop Science 44, 2049–2053.
Swindell, W.R., Bouzat, J.L., 2006. Gene flow and adaptive potential in *Drosophila melanogaster*. Conservation Genetics 7, 79–89.
Uselman, S.M., Snyder, K.A., Leger, E.A., Duke, S.E., 2014. First-year establishment, biomass and seed production of early vs. late seral natives in two medusahead (*Taeniatherum caput-medusae*) invaded soils. Invasive Plant Science Management 7, 291–302.
Uselman, S.M., Snyder, K.A., Leger, E.A., Duke, S.E., 2015. Emergence and early survival of early versus late seral species in Great Basin restoration in two different soil types. Applications in Vegetation Science 18, 624–636.
Vallentine, J.F., 1989. Range development and improvements. 3rd ed. 524. Academic Press, Cambridge, MA, USA.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, L., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23, 4407–4414.
Whalley, R.D.B., Jones, T.A., Nielson, D.C., Mueller, R.J., 1990. Seed abscission and retention in Indian ricegrass. Journal of Range Management 43, 291–294.
Wright, S.I., Bi, I.-V., Schroeder, S.G., Yamasaki, M., Doebley, J.F., McMullen, M.D., Gaut, B.S., 2005. The effects of artificial selection on the maize genome. Science 308, 1310–1314.