Oecologia (2003) 137:216–225
DOI 10.1007/s00442-003-1332-2

POPULATION ECOLOGY

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# The relative importance of sexual reproduction versus clonal spread in an aridland bunchgrass

Received: 15 November 2002 / Accepted: 28 May 2003 / Published online: 29 July 2003


**Abstract** *Festuca idahoensis* (Idaho fescue) is a perennial caespitose grass, common in semi-arid rangelands of the Intermountain West. To determine how individuals are recruited into a population, we studied two long-term monitoring plots that were established in 1937 at the Northern Great Basin Experimental Range in southeastern Oregon. The plots measured 3.05×3.05 m, and were located approximately 30 m apart. One plot was ungrazed, the other was subject to moderate levels of cattle grazing. The number of *F. idahoensis* plants in both plots increased ten-fold between 1937 and 1996, but whether this was due primarily to reproduction by seed or clonal fragmentation was unknown. In 1996, we mapped and sampled 160 plants of *F. idahoensis*. We used dominant inter-simple sequence repeat (ISSR) markers and codominant allozyme markers in order to identify genetic individuals and measure genetic diversity. Both plots were characterized by high levels of genetic and clonal diversity. When information from ISSRs, allozymes and sample location were combined, 126 genets were recognized, each consisting of one to four samples (ramets). By measuring the diameter of clones surrounding plants that were present in 1937, we estimated that clonal spread occurred at a rate of approximately 3.7 cm per decade, and thus was of secondary importance in the maintenance and increase of *F. idahoensis* stands. Sexual reproduction, rather than clonal fragmentation, accounted for most of the recruitment of new plants into these plots. The grazed plot had fewer ramets, genotypes, and clones than the ungrazed plot, but the ramets were significantly larger. Levels of genetic diversity did not differ in the grazed and ungrazed plots, but there was some evidence for a small, but significant level of genetic differentiation between the two. The results also indicate that *F. idahoensis* has the potential to be a long-lived species with some individuals persisting in excess of 60 years. This study demonstrates how long-term monitoring can be supplemented by genetic analysis to obtain detailed information on the population dynamics of plants. In the case of this community dominant species, this provides essential information for understanding succession and developing management and restoration strategies.

**Keywords** Allozyme · *Festuca idahoensis* · Idaho fescue · Inter-simple sequence repeat · Intermountain grassland

**Electronic Supplementary Material** Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00442-003-1332-2

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## Introduction

Many graminoid species are capable of both sexual and vegetative reproduction. Grasses in the Great Plains of North America generally rely heavily on vegetative reproduction (Briske and Richards 1995), often forming dense sods from rhizomes or stolons. Caespitose grasses (bunchgrasses) of the Intermountain West lack the rhizomes or stolons characteristic of sod-forming grasses. Caespitose grass populations are generally assumed to rely on recruitment from seed, although the size of individual plants increases via tiller growth (vegetative

reproduction). Long-lived caespitose grasses may eventually form "dead centers" (also referred to as "hollow crowns") resulting from ramet recruitment at the clone periphery and reduced axillary bud formation in the clone interior (Briske and Derner 1998). The eventual fragmentation of senescent caespitose grasses commonly results in physiologically separate, but genetically identical, clones (Watkinson and White 1986; Briske and Derner 1998).

Existing studies suggest variable potential outcomes of vegetative fragmentation on recruitment and clonal movement in grass populations. Fragmentation is a well-documented response to grazing pressure, and may have negative impacts on populations (Butler and Briske 1988; Briske and Richards 1995). In contrast, fragmentation may be advantageous to perennial grasses of arid western U.S. rangelands, where seedling recruitment is episodic and infrequent. For example, in the Jornada Experimental Range in southern New Mexico, *Bouteloua eriopoda* seedlings occurred in only 7 years between 1915 and 1968 (Neilson 1986). Fragmentation may increase the number of ramets (but not genotypes) within a caespitose grass population despite limited seedling recruitment. Previous research in more mesic environments has suggested, as a result of detailed morphological analysis, that widespread clonal movement occurs in caespitose grasses such as *Festuca ovina* (Harberd 1962) and *Holcus mollis* (Harberd 1967). However, assessing the importance of this process by examining morphology is difficult because morphology cannot reliably differentiate between genets and ramets. In addition, understanding of the importance of clonal fragmentation requires detailed demographic information over an extended period of time.

To better understand the population dynamics of clonal plants, genetic methods have been used to fingerprint individuals in order to differentiate between genets and ramets. Allozymes (reviewed in Ellstrand and Roose 1987; see also Parks and Werth 1993; Montalvo et al. 1997; Mayes et al. 1998; Pappert et al. 2000), anonymous DNA markers (Hsiao and Rieseberg 1994; Stewart and Porter 1995; Stiller and Denton 1995; Steinger et al. 1996; Olfelt et al. 1998; Suzuki et al. 1999; Corradini et al. 2002) and a combination of both methods (Waycott 1995; Ayres and Ryan 1997; Esselman et al. 1999; Ge and Sun 1999) have been effectively used in studies of clonal plant populations. Molecular methods are also valuable tools for identifying levels of genetic variation within and among plant populations, providing information that can be used to infer their reproductive systems and demographic histories. We chose to employ both dominant (ISSR) and codominant (allozyme) markers. The hypervariable ISSR markers increase our ability to detect unique genotypes, while the allozyme markers allow testing of Hardy-Weinberg equilibrium in the sampled population.

*Festuca idahoensis* (Idaho fescue), is a long-lived perennial caespitose grass, common in semi-arid intermountain grasslands of North America. It is a member of Section Ovinae (the fine fescues), an apparently monophyletic group (Gaut et al. 2000). We examined a population of *F. idahoensis* at a long-term monitoring site in southeastern Oregon, USA. In the late 1930s, a grazing exclosure was established at this location. In 1937, individual plants were identified and mapped within plots both inside (ungrazed) and outside (grazed) the exclosure. This situation provided a unique opportunity to combine historical vegetation maps and modern genetic marker techniques in order to investigate (1) the relative importance of clonal fragmentation and sexual reproduction on the population dynamics of a caespitose grass of semi-arid rangelands, (2) the levels and distribution of genetic diversity, and (3) the effects of long-term grazing on these population processes.

## Materials and methods

### Study site and plant collection

The study site was located at 43°31′48.715″N, 119°40′43.842″W on the Northern Great Basin Experimental Range in Harney County, Oregon, USA. The vegetation at this site is a shrub steppe plant community with a sparse juniper overstory; dominant plants include *Artemesia tridentata*, *Festuca idahoensis*, and *Pseudoroegneria spicata* (Lentz and Simonson 1986). As part of a long-term monitoring experiment initiated in 1937, all plants were identified and mapped in a pair of 3.05×3.05 m permanent plots, located approximately 30 m apart. Original maps are archived at the Eastern Oregon Agricultural Research Center, Burns, Oregon. One plot was subjected to annual moderate to heavy grazing since that time. The sampling area was near a road and a watering point, and thus received heavier grazing pressure than the 800 ha pasture as a whole. A continuously maintained fence excluded grazing from a 2 ha area including the second plot. On 2 July 1996, samples consisting of leafy tillers were taken from all *F. idahoensis* plants in the two permanent plots. In order to minimize disturbance to the long-term monitoring plots, relatively small amounts of plant material were collected. A voucher specimen was collected and deposited in the OSU Herbarium (Liston 1024). Samples from the grazed plot were numbered 101–172; those from the ungrazed plot were numbered 200–294. Discrete, caespitose clumps were sampled once; four linear groups (where several caespitose clumps were distinguishable but contiguous) were sampled more than once (samples 130, 131; 168, 169, 170; 272, 273, 275; 276, 277, 278). A total of 167 samples were transported to Oregon State University on ice. One ramet (samples 141 and 153) was inadvertently sampled twice; it is treated as a single sample in the analyses. ISSR data are missing for nine samples (two in the grazed and seven in the ungrazed plot, Figs. 1, 2) due to inadequate plant material. Sample 126 had an unusual ISSR banding pattern; it was determined to be a species of *Achnatherum*, and was excluded from subsequent analyses.

At the time of collection, clear Mylar sheets were placed over the plots, and the location and outlines of all *F. idahoensis* plants were recorded. The initial maps were digitized using Tsoft Digi-Edit (Jones 1994). The 1996 maps were compared to the 1937 maps, and the positions of plants mapped in 1937 ("old plants", Figs. 1, 2) were relocated.

### Genetic methods

We used both inter-simple sequence repeat (ISSR) markers (Gupta et al. 1994; Zietkiewicz et al. 1994; Wolfe and Liston 1998) and allozyme methods to genotype plants. We also used the ISSR markers to estimate population genetic parameters. Like random

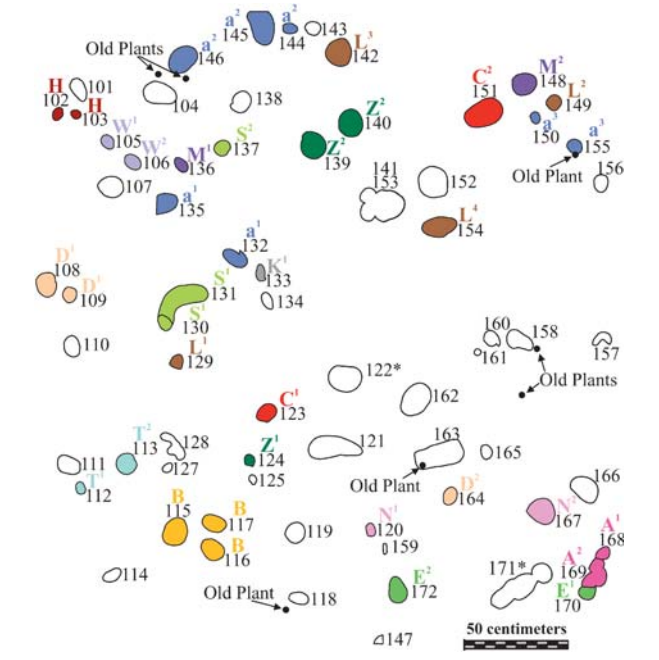


**Fig. 1** Grazed plot. Outlines of all *Festuca idahoensis* plants with sample numbers. Plants of the same color and letter code share an ISSR genotype. *Superscripts* following the letter codes represent distinct allozyme genotypes. *Uncolored plants* have unique ISSR genotypes. Plants marked by an *asterisk* were not sampled for ISSR variation. *Old plant* indicates the location of an individual mapped in 1937

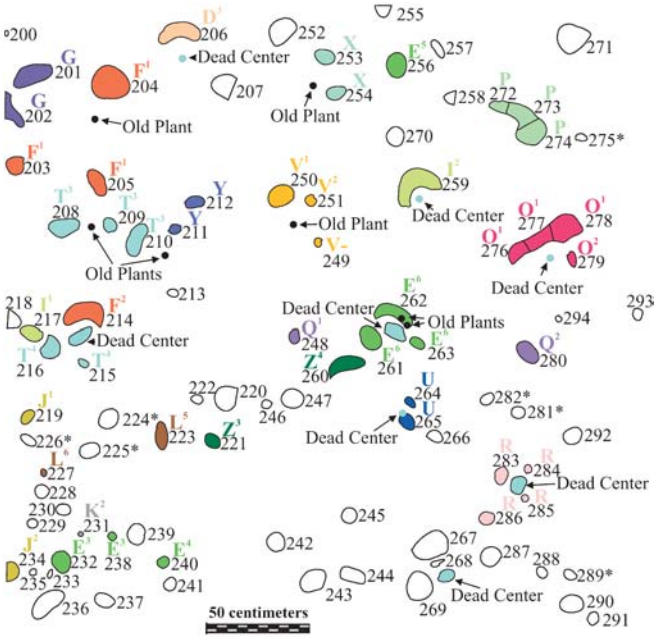


**Fig. 2** Ungrazed plot. Outlines of all *F. idahoensis* plants with sample numbers. Plants of the same color and letter code share an ISSR genotype. *Superscripts* following the letter codes represent distinct allozyme genotypes. *Uncolored plants* have unique ISSR genotypes. Plants marked by an *asterisk* were not sampled for ISSR variation. *Old plant* indicates the location of an individual mapped in 1937

amplified polymorphic DNA (RAPD), ISSRs are anonymous PCR-based dominant markers. In contrast to RAPDs, ISSR primers are longer, and associated with simple sequence repeat (microsatellite) motifs. The ISSR method tends to be more robust to variation in experimental conditions (e.g. DNA amount and quality) and produce higher levels of variation than RAPDs (Wolfe and Liston 1998).

Methods of ISSR analysis followed Camacho and Liston (2001). Genomic DNA for each individual was extracted from 10–50 mg of leaf tissue using the method of Doyle and Doyle (1987), modified for smaller samples. Excess leaf tissue was stored at –20°C for future extractions. Each ISSR reaction was carried out in a total volume of 17 $\mu$l, containing a 1.0 M TRIS-HCl, pH 9.0 and 0.4 M ammonium sulfate buffer and one unit of *Tfl* polymerase (Epicentre, Madison, Wis.), 5.3 $\mu$l double deionized water, 1 $\mu$l BSA (1 mg ml$^{-1}$), 1 $\mu$l of primer (10 nmol ml$^{-1}$), and 10–20 ng of genomic DNA (1 $\mu$l volume). Initial denaturation was carried out for 1 min at 94°C, followed by 34 cycles of 45 s at 94°C, 30 s at 50°C, 15 s at 72°C, and a final 5 min extension at 72°C. ISSR primers were obtained from the University of British Columbia Biotechnology Laboratory. PCR reactions were performed in an MJ-Research (Watertown, Mass.) PTC–100 thermocycler. Products were analyzed on 1.2% agarose gels in 1X TBE buffer, and stained with ethidium bromide. Band size was estimated from a 100 bp ladder (New England Biolabs, Beverly, Mass.). Loci were named based on the primer and observed band size. Sixty ISSR primers were screened for band number and informative variation in *F. idahoensis*. Primers 811 [$(GA)_8C$], 835 [$(AG)_8YC$], and 842 [$(GA)_8YG$] were selected for this study. Band reproducibility was determined by running samples in duplicate.

Leafy tillers that had been stored at –20°C as backup for DNA extraction were subsequently used for allozyme analysis. Excess material was not available for all plants. Leaves were ground in a TRIS citrate buffer, pH 7.5 (Gottlieb 1981) and absorbed onto wicks prepared from Whatman 3 M chromatography paper. Methods of electrophoresis and staining followed Wendel and Weeden (1989). A histidine citrate electrode and gel buffer (pH 5.7) (Soltis et al. 1983) was used to resolve phosphoglucomutase (PGM). A lithium borate electrode buffer (pH 8.3) and TRIS citrate—lithium borate gel buffer (pH 8.3) (Soltis et al. 1983) were used to resolve phosphoglucose isomerase (PGI) and triosephosphate isomerase (TPI).

## Data analyses

*F. idahoensis* has a chromosome number of $2n$=28 and is considered an allopolyploid (Darlington and Wylie 1955). Allopolyploids are generally characterized by disomic inheritance (Soltis and Soltis 2000). Consistent with this, allozyme studies have found disomic inheritance for all examined loci in *F. idahoensis* (Wilson 1999; Samman et al. 2000). Furthermore, cytological and crossing studies have found evidence for multivalent suppressors in polyploid *Festuca* species (Jauhar 1975; Stace and Ainscough 1984). Based on these findings, we have analyzed the allozyme and ISSR loci with standard genetic methods that assume disomic inheritance.

The dimeric enzymes, cytosolic PGI2 and TPI2, both appear to be coded for by two homoeologous loci. All variation in TPI and nearly all variation PGI were assigned to one of the two homoeologous loci. We tested the hypothesis of Hardy-Weinberg equilibrium, with duplicate samples of a genet removed, using POPGENE vers. 1.32 (Yeh et al. 1997). Neither locus differed significantly from Hardy-Weinberg equilibrium (PGI2: $n$=88, $\chi^2$=3.592, $P$=0.31; TPI2: $n$=92; $\chi^2$=5.424, $P$=0.14). Therefore, we assumed Hardy-Weinberg equilibrium for genetic analysis of ISSR loci. Variation at the monomeric enzyme, PGM1, could not be partitioned among the putative homoeologous loci, and thus single locus statistics could not be accurately calculated. However, this locus, in addition to the other two, was useful in identifying genets.

ISSR data were scored as presence (1) and absence (0) of bands. Genetic variation was quantified as percent polymorphic loci ($P$),

mean expected heterozygosity ($H_e$, averaged across loci), and total heterozygosity ($H_T$). For expected heterozygosity from the dominant ISSR markers, we used the method of Lynch and Milligan (1994), pruning all loci for which the null allele frequency was less than 3/N. Hardy-Weinberg equilibrium was assumed (see Results) in the calculation of ISSR allele frequencies used in estimating $H_e$. The probability of occurrence of a multilocus ISSR genotype (assuming no genetic linkage) was determined following Parks and Werth (1993) and Montalvo et al. (1997).

We determined the magnitude of genetic differentiation ($G_{ST}$) between the plots using both the mean $G_{ST}$ across all polymorphic ISSR loci (Hamrick and Godt 1989) and the mean estimates of $H_e$ and $H_T$ (Nei 1973). The significance of the difference between plots in mean $H_e$ and in $G_{ST}$ was evaluated with a Monte Carlo randomization procedure using Resampling Stats for Excel (Blank 2001). We randomly assigned (without replacement) the ISSR multilocus genotypes to two groups equaling the size of the two plots. The values of $G_{ST}$ were calculated 1,000 times for each group, using (1) all sampled individuals, and (2) duplicate samples of a genet (defined below) removed. The observed values were compared with the Monte Carlo distribution. The null hypothesis, that the observed values were due to chance, was rejected when the observed value was more extreme than the 95% confidence interval for the Monte Carlo distribution (i.e. above the 25th or below the 976th ranked Monte Carlo value). Gene flow estimates ($Nm$) used the method of Wright (1951).

We defined genets as samples or sets of samples that had a single ISSR genotype, occurred in the same plot, were not separated by plants with different ISSR genotypes, and shared the same allozyme genotype. Clonal diversity statistics include Simpson's diversity index (a measure of multilocus diversity), evenness, and the number of distinct genotypes relative to the number of samples (Ellstrand and Roose 1987; Montalvo et al. 1997). For Simpson's diversity index ($d$), we used the equation for finite populations (Pielou 1969), $d=1-\sum [n_i(n_i-1)]/[N(N-1)]$, where $N$ = number of samples and $n_i$ = number of samples of genotype $i$. Evenness ($E$), is a measure of the equality of distribution of genotypes, calculated as $E = d/d_{max}$, where $d_{max}$ is the maximum value the Simpson Index can attain; i.e. $[(k-1)N] / [k(N-1)]$ , where $k$ = the number of genets observed.

The hypothesis that nearby samples were more genetically similar was tested within each plot. Pairwise geographic distances between samples were generated using Idrisi for Windows (Eastman 1997) and Dice similarity $[S=2x / (2x+y)]$, where $x$ = bands shared between samples and $y$ = unshared bands, present in only one sample) was calculated from ISSR genotypes in NTSYS-pc Vers. 2.02 (Rohlf 1997). The matrices were compared with a Mantel test as implemented in NTSYS-pc. Chi-square and Student's $t$-tests were calculated using Statgraphics Plus Vers. 3.0 (1997).

## Results

### Clone recognition and abundance

The three ISSR primers used generated a total of 21 markers, 18 (86%) of them polymorphic. Among 156 samples, these markers revealed 66 genetically unique samples (solitary genets) and 90 samples that shared 27 genotypes (Table 1). The identity of 68 samples sharing 20 ISSR genotypes was further evaluated using three allozyme loci. Lack of material prevented testing of ISSR groups C, H, I, M, N, Q, and U. Allozyme results revealed that seven ISSR groups (26% of all groups; 35% of those tested with allozymes) each represented a single genet. Group S was uniform for allozymes, but was separated into two clones based on spatial separation (Fig. 1).

**Table 1** Number of plants observed, DNA samples, genets, and genetically unique ramets of *Festuca idahoensis* in the long-term monitoring plots. *Solitary genet* A genotype or ISSR genotype represented by only one sample. The number of ISSR groups and total genets are not additive due to six genotypes that are shared across plots

| | Total | Grazed plot | Ungrazed plot |
|---|---|---|---|
| Plants observed | 160 | 69 | 91 |
| DNA samples | 156 | 68 | 88 |
| Based on ISSRs only | | | |
| Solitary genets | 66 | 28 | 38 |
| Ramets that share ISSR genotypes | 90 | 40 | 50 |
| ISSR groups | 27 | 15 | 18 |
| Total genets | 93 | 43 | 56 |
| Based on ISSRs, allozymes, and spatial separation | | | |
| Solitary genets | 105 | 50 | 55 |
| Ramets that comprise a clone | 51 | 18 | 33 |
| Total genets | 126 | 58 | 68 |

Thirteen ISSR genotype groups (48% of all groups; 65% of those tested) included samples with two or more allozyme genotypes (Electronic Supplementary Material, Appendix 1).

In general, ISSR genotype groups that included many samples had unique genotypes with high probabilities of occurrence (Electronic Supplementary Material, Appendix 2). Of the ten ISSR genotypes that had a probability of second occurrence greater than 0.60, eight were represented by two or more samples, and all eight of these ISSR groups were represented by two or more clones. Six ISSR genotypes (D, E, K, L, T, and Z) occurred in both plots. Four of these had a probability of second occurrence greater than 0.60. With a probability of 0.20, the ISSR genotype T was the least probable genotype that occurred in several samples and both plots. All six ISSR genotype groups that occurred in both plots and were also tested with allozymes included two or more allozyme genotypes. Therefore, all samples that shared an ISSR genotype but occurred in different plots were treated as different genets in the analyses.

Within plots, the geographic and genetic distances (calculated using ISSR genotypes) between plants were not related (grazed: $r$=0.08, Mantel $Z$=70,192, $t$=3.4805, $P$=0.9997; ungrazed: $r$=0.14, Mantel $Z$=121,875, $t$=6.2085, $P$=1.0000). However, in many instances the spatial arrangement of plants was related to genetic similarity, as seen in the proximity of ramets comprising putative clones (Figs. 1, 2). In the case of the widespread ISSR genotypes E and T, allozyme data divided them into small, geographically coherent groups that likely are genets (Figs. 1, 2). Three of the four linear clumps sampled at least twice turned out to share ISSR genotypes and allozyme genotypes; these constitute ISSR group P (Fig. 2) and parts of ISSR genotype groups O and S (Figs. 1, 2). However, one such linear clump consisted of three genetically distinct individuals (sample 170 of ISSR group E plus samples 168 and 169 of ISSR genotype

**Table 2** Geographic distances between the 1937 locations of *F. idahoensis* plants or currently observed "dead centers" and surrounding ramets present in 1996. Clone E6 was included in both categories

| ISSR genotype | Sample number | Distance to site of 1937 plant (cm) | Distance to dead center (cm) | Rate of spread from 1937 plant (cm decade$^{-1}$) | Rate of spread from dead center (cm decade$^{-1}$) |
|---|---|---|---|---|---|
| E6 | 261 | 19 | 8 | 3.17 | 1.33 |
| E6 | 262 | 5 | 7 | 0.83 | 1.17 |
| E6 | 263 | 15 | 6 | 2.50 | 1.00 |
| F1 | 203 | 47 | | 7.83 | |
| F1 | 204 | 20 | | 3.33 | |
| O1 | 276 | | 16 | | 2.67 |
| O1 | 277 | | 10 | | 1.67 |
| O1 | 278 | | 16 | | 2.67 |
| R | 283 | | 6 | | 1.00 |
| R | 284 | | 5 | | 0.83 |
| R | 285 | | 3 | | 0.50 |
| R | 286 | | 19 | | 3.17 |
| T3 | 208 | 14 | | 2.33 | |
| T3 | 209 | 10 | | 1.67 | |
| T3 | 210 | 24 | | 4.00 | |
| T4 | 215 | | 16 | | 2.67 |
| T4 | 216 | | 14 | | 2.33 |
| U | 264 | | 5 | | 0.83 |
| U | 265 | | 3 | | 0.50 |
| V- | 249 | 15 | | 2.50 | |
| V2 | 251 | 15 | | 2.50 | |
| X | 253 | 16 | | 2.67 | |
| X | 254 | 13 | | 2.17 | |
| a2 | 144 | 60 | | 10.0 | |
| a2 | 145 | 48 | | 8.0 | |
| a2 | 146 | 10 | | 1.67 | |
| a3 | 150 | | 28 | | 4.67 |
| a3 | 155 | | 5 | | 0.83 |
| Mean and SD | | 22.1±16.2 | 10.4±7.0 | 3.68±2.70 | 1.74±1.18 |

group A, Fig. 1). Although most ISSR groups tested with allozymes consisted of two or more clones, only 22 clones were identified, each consisting of two to four samples (Figs. 1, 2).

Some plants shared such unusual patterns that if they were not clones, they were likely siblings. For example, plants with the uncommon ISSR genotype O share an uncommon PGM1 genotype and surround the site occupied by an *Festuca idahoensis* in 1937 (Fig. 2). Three of them formed a single linear clump and shared an allozyme genotype; the fourth member of this group grew only 16.8 cm from the linear clump but had a rare PGI2 genotype. The ISSR genotype V had a probability of second occurrence of only 0.034. Three plants with this genotype formed a "fairy ring", or extensive hollow crown, around the site of a 1937 plant, but these three have at least two different allozyme genotypes. The locations of samples in ISSR genotype groups V and O were consistent with the hypothesis that these genetically distinct plants might have established from seed near their parent plant.

## Abundance, clonal spread, and longevity

Many more *F. idahoensis* plants grew in the studied plots in 1996 than in 1937. In 1937, 8 plants were observed in the grazed plot. In 1996, 69 plants were observed. Inside the grazing exclosure, 7 plants were observed in the plot in 1937 and 91 in 1996. Over both plots, the proportion of samples distinguished as members of different genets is high (greater than 0.5), and the average number of samples per genet was a low 1.238 (Table 3). The distribution of samples among clones was very even ($E$=0.998) because most genets consisted of a single sample and none consisted of more than four. Overall, 80.8% of the samples represent unique genotypes ($k/N$, Table 3).

Seven clones (E6, F1, T3, V1 or V2, X, a2, a3) appeared to surround the location of plants present in 1937. If these clones were the products of vegetative growth from these 1937 plants, they spread at an average rate of 3.68 cm decade$^{-1}$ (SD=2.70, maximum=10.0 cm) (Table 2) and survived for at least 60 years. Five clones in the ungrazed plot (E6, O1, R, T4, and U) surrounded visible "dead centers" that are assumed to be their point of origin (Fig. 2). If so, they spread at a mean rate of 1.74 cm decade$^{-1}$ (SD =1.18 cm, maximum =4.67 cm), if they had been spreading linearly for the entire 60 years since the original maps were made (Table 2). Considering that the rate of spread calculated for these five clones surrounding "dead centers" is less than half that of the seven clones surrounding "old plants", it is likely that they were established much later than 1937.

This calculated rate of spread did not include samples that shared ISSR genotypes but did not grow adjacent to each other. In general, these widespread ISSR genotypes had high probabilities of second occurrence (Electronic

**Table 3** Clonal diversity statistics for *F. idahoensis*, compared to that reported for other fescues. *N* Number of samples, *G* number of distinct ISSR genotypes, *k* number of genets (considering ISSR, allozyme, and plot data for *F. idahoensis*, see footnotes for other studies), *G/N* "proportion distinguishable" (Ellstrand and Roose1987) using only ISSR data, *G/k* proportion of clones with unique ISSR genotype, = *G/n* of Montalvo et al. (1997), *k/N* "proportion distinguishable" (Ellstrand and Roose1987) using all data, *N/k* number of samples (ramets) per genet, *d* Simpson index, and *E* evenness

| | *N* | *G* | *K* | *G/N* | *G/k* | *k/N* | *N/k* | *d* | *E* |
|---|---|---|---|---|---|---|---|---|---|
| Overall | 156 | 99 | 126 | 0.635 | 0.786 | 0.808 | 1.238 | 0.997 | 0.998 |
| Grazed plot | 68 | 43 | 58 | 0.632 | 0.741 | 0.853 | 1.172 | 0.994 | 0.997 |
| Ungrazed plot | 88 | 56 | 68 | 0.636 | 0.824 | 0.773 | 1.294 | 0.993 | 0.996 |
| Other studies: | | | | | | | | | |
| *F. novae-zelandiae*[a] | 114 | | 69 | | | 0.605 | 1.652 | | |
| *F. rubra*[b] (Plot J) | 112 | | 32 | | | 0.286 | 3.500 | | |
| *F. rubra*[b] (Plot S) | 79 | | 27 | | | 0.342 | 2.926 | | |

[a] Lord (1993) *k* determined by morphology
[b] Suzuki et al. (1999) *k* determined by RAPDs. Four incompletely sampled plots are not included

**Table 4** Population genetic diversity statistics based on ISSR markers in *F. idahoensis*. *N* Sample size, *P* % of polymorphic loci, $H_e$ mean expected heterozygosity, following Lynch and Milligan (1994), $H_T$ total genetic diversity, $G_{ST}$ magnitude of genetic differentiation calculated as: (1) mean across loci, and (2) from mean heterozygosities

| | *N* | P (%) | $H_e$ | $H_T$ | Difference in $H_e$ | $G_{ST}$ 1 | $G_{ST}$ 2 |
|---|---|---|---|---|---|---|---|
| Grazed plot, all samples | 68 | 71.4 | 0.258 | | | | |
| Ungrazed plot, all samples | 88 | 81.0 | 0.263 | | | | |
| Total | 156 | 85.7 | | 0.263 | 0.00110 | 0.0187 | 0.0095 |
| Monte Carlo Rank | | | | | 558 *P*=0.88 | 988 *P*=0.024 | 960 *P*=0.08 |
| Grazed plot, duplicate genets removed | 58 | 71.4 | 0.261 | | | | |
| Ungrazed plot, duplicate genets removed | 68 | 81.0 | 0.272 | | | | |
| Total | 126 | 85.7 | | 0.270 | 0.00335 | 0.0167 | 0.0120 |
| Monte Carlo Rank | | | | | 400 *P*=0.80 | 995 *P*=0.01 | 933 *P*=0.134 |

Supplementary Material, Appendix 2), and comparing the recent and historical map provided no compelling evidence that disjunct samples might be members of the same clone (Figs. 1, 2). Therefore, applying these rates of spread to distant samples that shared ISSR genotypes would be inappropriate.

### Comparing grazed and ungrazed plots

The grazed plot contained fewer ramets, fewer different genotypes, and fewer clones than the ungrazed plot (Table 1). However, the total basal area coverage of *F. idahoensis* was similar in both plots (grazed =0.653 $m^2$; ungrazed =0.668 $m^2$). The average plant size was 0.92 $cm^2$ in the grazed plots and 0.70 $cm^2$ in the ungrazed plot, this size difference was significant ($t$=2.270, $P$=0.0245). The ungrazed plot had eight visible dead centers, while the grazed plot had none (Figs. 1, 2). The grazed plot had fewer samples per clone (*N/k*) than the ungrazed one (Table 3). The maximum number of samples per clone was three in the grazed and four in the ungrazed plot, and clonal evenness was virtually identical in the two plots (Table 3).

Measures of genetic diversity were calculated from ISSR genotypes (Table 4). The difference in number of polymorphic loci in the grazed and ungrazed plots was not significant ($\chi^2$=0.131, $P$=0.717). Using the criteria of Lynch and Milligan (1994), six loci were excluded from the calculations of $H_e$ in the grazed plot; five loci were excluded for the ungrazed plot and total population. Based on the Monte Carlo randomized replicates, the average difference in mean $H_e$ was not significant between the two plots (Table 4). The estimates of genetic differentiation were significant ($P$ <0.05) when $G_{ST}$ was calculated following Hamrick and Godt (1989), but less so ($P$<0.10) when calculated following Nei (1973). In all cases, the estimates of $G_{ST}$ were low, and consequently estimated gene flow ($N$m) was high (13.13–26.00). Similar results were obtained when duplicate genets were excluded from the calculations (Table 4).

## Discussion

Long-term monitoring can provide important insights into the temporal dimension of ecological change. The most valuable results can be obtained from the oldest monitoring sites. Unfortunately, older sites often lack detailed documentation and may not be consistently maintained over the decades. The permanent plots established at the Eastern Oregon Agricultural Research Center were

meticulously measured in 1937 and well-maintained over 6 decades. However, since the distribution of individual plants was not monitored between 1937 and 1996, we chose to employ genetic methods to reconstruct the population history. The combined use of detailed mapping of individual plants and genetic methods allowed us to determine that sexual reproduction rather than clonal spread was the primary mechanism by which *Festuca idahoensis* numbers and density increased at this site over the past 60 years. Our conclusions are based on results from a single site, and opportunities to replicate them at other locations are limited by the lack of appropriate long-term monitoring experiments. Nevertheless, the study site is typical of Idaho fescue communities throughout the region, and the levels and distribution of genetic diversity observed are similar to other studies of this species (Wilson 1999; Samman et al. 2000). For these reasons, we believe that it is reasonable to assume that our conclusions are applicable to other populations of *F. idahoensis* in the Northern Great Basin.

## Recruitment

Considering the high number of genotypes detected in this study, most of the new *F. idahoensis* individuals (ramets) which had established since 1937 are assumed to be the result of sexual reproduction via seed. Apomictic reproduction is unknown in the genus *Festuca* (Auquier 1977) and in section Ovinae in particular (C.A. Stace, University of Leicester, personal communication). A minimum of 63.5% of the sampled *F. idahoensis* plants were genetically distinct, based on unique ISSR genotypes. This is remarkably similar to the 66% unique genotypes among *Schizachyrium scoparium* plants (also a caespitose grass) sampled in grazed and ungrazed populations (Carman and Briske 1985). Taken into account allozyme genotypes and considering plants in separate plots to be independently derived, 80.8% of the sampled plants are genetically distinct. In considering these results, two sources of potential error must be taken into account: (1) inclusion of additional ISSR loci would likely reveal additional differences among individuals that were indistinguishable here, and (2) there is the potential for somatic mutations to occur in long-lived plants (Corradini et al. 2002), which would result in non-recognition of clonal fragments. Although these factors could change the assignment of individual plants to ramets or genets, they would not alter the overall conclusions reported here.

Combined ISSR, allozyme, and spatial data revealed a minimum of 126 different genets in the 18.6 $m^2$ area sampled. This average density of 6.8 different genotypes per each square meter is also consistent with a predominance of sexual reproduction. However, we were also able to identify groupings that likely represent genetic individuals that had fragmented and formed multiple clumps. Comparing individual plant locations from 1937 with those in 1996, we estimated that the rate of clonal spread was approximately 3.7 cm per decade. This slow rate of clonal spread is consistent with the caespitose growth form of *F. idahoensis* (Conrad and Poulton 1966; Jaindl et al. 1994). Our results are in contrast to those of Harberd (1962, 1967), who found greater rates of clonal spread in the caespitose grasses *F. ovina* and *F. mollis.* Likewise, Hartnett (1993) documented rates of 12.3 cm per year in *Panicum virgatum*, a rhizomatous species of tallgrass prairie. These studies were done in mesic climatic conditions, whereas our study was done in a semi-arid environment. The distribution of samples among clones in *F. idahoensis* indicated that *F. idahoensis* spreads clonally somewhat less than does the tussock-forming *F. novae-zelandiae* (Lord 1993, Table 3). Clone diversity statistics for *F. idahoensis* differed greatly from those reported for *F. rubra*, a rhizomatous species which spreads vegetatively to an important extent (Suzuki et al. 1999, Table 3). Clonal fragmentation and movement do not appear to contribute greatly to increased plant density in *F. idahoensis.* We conclude that most density changes of *F. idahoensis* are a result of seedling recruitment, although fragmentation may enhance the persistence of this species.

## Longevity

Seven individuals present in 1937 appeared to persist over the 60-year period. Our estimate is based upon the location of a 1937 plant and the identification of clones that had apparently fragmented away from the original plant. This represents a minimum age, because the individuals were monitored only in 1937 and 1996. The 60-year-old genetic individuals showed no evidence of senescence at the time of this study. This longevity is consistent with that calculated for other caespitose grasses. The life expectancy for *Pseudoroegneria spicata* (bluebunch wheatgrass, often found growing with *F. idahoensis* in the same habitat) has been estimated as 41–43 years (West et al. 1979) or 65 years (Treshow and Harper 1974). Individuals of *Deschampsia caespitosa* are estimated to survive 25–60 years (Gatsuk et al. 1980). In contrast, the maximum age calculated for tussock-forming *F. novae-zelandiae* is 500 years (Lord 1993), while a large clone of the vegetatively spreading *F. rubra* was estimated to be at least 400 and perhaps over 1,000 years old (Harberd 1961).

## Grazed versus ungrazed

Since 1937, a 10-fold increase in numbers of *F. idahoensis* individuals was observed on both the grazed and ungrazed plots. This increase can be viewed in the context of changing grazing patterns in the region. During the late 1800s and early 1900s livestock use of western rangeland was essentially unregulated, with severe declines occurring to palatable bunchgrasses such as *F. idahoensis* (Mack and Thompson 1982). Establishment of the Northern Great Basin Experimental Range brought an

end to unregulated grazing on the study area. Although cattle grazing did continue, its severity was never as great as that which occurred during the prior decades. Previous research has indicated that *F. idahoensis* to be relatively grazing tolerant in comparison to other indigenous bunchgrasses of the sagebrush grassland (Jaindl et al. 1994). The grazing tolerance of this species coupled with lower grazing pressures were most likely factors contributing to the population increase on the study site since the original census in 1937.

We documented more clonal fragmentation (Tables 1, 3) in the ungrazed plot, apparently due to outward spread and fragmentation of the original bunchgrass. Presumably, litter accumulation in the center of the plant reduces tiller development either through mulching effects or a decrease in phytochrome stimulated tillering (Briske and Derner 1998). Over time, migration of tillers outward and away from the plant center leads to fragmentation of the original clump. Grazing may actually prevent fragmentation as it would tend to reduce litter accumulation in the clump. Consistent with this, we found more plants with dead centers in the ungrazed area relative to the grazed area. Individual plants were significantly smaller in the ungrazed plot, and we suspect that fragmentation may be part of the explanation.

We observed no significant differences in the levels of genetic diversity ($P$ and $H_e$) between the grazed and ungrazed plots. This is consistent with prior studies that have failed to observe an impact of grazing on neutral genetic variation in perennial grasses (Carman and Briske 1985; Tómas et al. 2000; Matlaga and Karoly 2003). In our study, we also examined the effect of excluding duplicate genets from the calculations. The results did not change, suggesting that differences in the amount of clonal fragmentation have not resulted in observed changes in genetic diversity.

In contrast to the results for genetic diversity, we observed a small, but significant level of genetic differentiation, when $G_{ST}$ was calculated as an average of all polymorphic loci ($P$=0.024). However, the estimate of $G_{ST}$ was less significant ($P$=0.08) when calculated as defined by Nei (1973). Culley et al. (2002) demonstrated that the two methods rarely give the same value of $G_{ST}$, but that many researchers are not aware of this phenomenon. In our study, the differences in the estimate of $G_{ST}$ were relatively small (Table 4), but nevertheless resulted in different levels of statistical significance in the Monte Carlo randomizations. Interestingly, Matlaga and Karoly 2003 found no evidence of genetic differentiation in *F. idahoensis* at a different pair of grazed and ungrazed plots at the Northern Great Basin Experimental Range using similar genetic and analytical methods. Their study differed from ours in using ISSR loci only, not mapping plant locations, and not taking into account the 1937 data. We propose that founder effects and genetic drift resulting from the large increases in population size documented here could be responsible for the genetic differentiation observed in our study. Idaho fescue is wind-pollinated, and gene flow through pollen would be expected to reduce genetic differentiation. However, this is balanced by the observation that seed dispersal may be limited, as inferred from the proximity of closely related individuals.

## Conclusions

Sexual reproduction, rather than clonal spread, produced most of the *F. idahoensis* plants observed in this study. However, species with significant clonal spread are sometimes highly variable (Ellstrand and Roose 1987; Lehmann 1997). Populations of clonally spreading *F. rubra* are often variable (Harberd 1961; Harberd and Owen 1969; Skálová et al. 1997). Variability itself does not rule out a significant role for clonal spread in *F. idahoensis*, but additional evidence for the predominance of sexual reproduction is provided by the extremely slow rate of clonal spread observed here. Even though seven plants apparently persisted during the entire 60 years between sampling, their clonal spread could not cover a large part of the study area nor account for the many additional individual observed in 1996, as compared to 1937.

Overall, clonal fragmentation appeared to be of secondary importance as a mechanism of *F. idahoensis* propagation. Sexual reproduction was the principal mechanism for establishment of new individuals on the study sites. This leads to an important recommendation if grazing is to be compatible with the preservation of this species on Intermountain rangelands. Because sexual reproduction is most likely to be episodic in nature, to establish new individuals of *F. idahoensis* in an area requires careful attention to allowing for seed production and seedling establishment during favorable climatic periods. This point is especially critical in habitat restoration, although because of the long-lived nature of the species, less so in stands that have high population densities of Idaho fescue.

**Acknowledgements** We thank David Courtney, Uzi Plitmann, and Jeff Rose for assistance with sampling and field mapping. David Briske, Susan Kephart, Keith Karoly, and two anonymous reviewers provided valuable comments on the manuscript. This study was funded by the USDA Agricultural Research Service.

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