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Genetic Diversity in the Introduced Clonal Grass *Poa bulbosa* (Bulbous Bluegrass)

Abstract

Bulbous bluegrass (*Poa bulbosa*) is a perennial bunchgrass with a widespread distribution throughout its native range in Europe and the Mediterranean. This grass has been introduced into North America and now occurs throughout much of the western United States. Within its native range, bulbous bluegrass reproduces mainly through sexual means; however, in North America clonal reproduction occurs primarily through the production of vegetative structures called bulblets. High chromosome counts are frequently reported for this species and suggest it is a polyploid. To assess the level and pattern of genetic diversity of bulbous bluegrass across a portion of its introduced range, a total of 10 populations from Idaho, Oregon, and Washington were analyzed by staining for 14 enzymes that were coded for by 19 genetic loci. Results indicate that bulbous bluegrass contains higher levels of genetic variation than expected for an introduced clonal plant species: 27.9% of loci are polymorphic per population, with an average of 1.54 alleles per locus, and a mean observed heterozygosity of 0.202. On average, 64% of all individuals within these populations possess three or four alleles at one or more loci; a value consistent with previous reports for autopolyploid species. A total of 84 multilocus genotypes were detected in these 10 populations, with an average of 9.6 genotypes per population. The number and complex distribution of multilocus genotypes observed in this study may be the result of multiple introductions of this species into its new range, and/or occasional sexual reproduction within introduced populations.

Introduction

Biological invasions are defined as the successful introduction and establishment of an organism into a region or habitat that was previously unoccupied by that species (Mack 1985). Biological invasions can vary geographically from simple range extensions to intercontinental migration (Barrett and Richardson 1986). Successful invaders (whether they be plants or animals) often possess the ability to aggressively colonize a new territory, with rapid range expansion across the introduced region (Mack 1985, Barrett and Richardson 1986, Barrett and Shore 1989). Once invasions occur, they can pose ongoing problems because they can act as vectors for disease, be expensive, alter ecosystem processes, and reduce biological diversity (Vitousek et al. 1996). In addition, several recent reviews have focused on the issue of biological invasions as forces for global change (D'Antonio and Vitousek 1992, Vitousek et al. 1996). Additionally, invasive species provide biologists with excellent model systems for genetic and evolutionary studies (Baker and Stebbins 1965, Barrett and Husband 1990). Invasive plant species are commonly reported to possess uniparental reproductive systems, be polyploids, contain depauperate levels of genetic variation within populations, have high levels of genetic differentiation, and exhibit multilocus structure among introduced populations (Baker 1974, Brown and Marshall 1981, Barrett and Richardson 1986).

Traditionally, introduced populations are believed to be established by only a small number of immigrants, often resulting in a loss of genetic variation through sampling effects (Barrett and Richardson 1986, Barrett and Shore 1989). In addition to founder effects and genetic bottlenecks, an absence of repeated immigration events will contribute to high levels of genetic differentiation among populations (Brown and Marshall 1981, Wade and McCauley 1988). Conversely, multiple introductions of different genotypes at the same or nearby locations can result in greater genetic variation in founder populations (Barrett and Husband 1990). Recent genetic evidence for cheatgrass (*Bromus tectorum* L.) and white bryony (*Bryonia alba* L.) suggests that multiple introductions of alien species may be more common than previously thought (Novak et al. 1991, Novak and Mack 1993, Novak et al. 1993, Novak and Mack 1995).

Uniparental reproductive systems, such as self-pollination or clonal reproduction (i.e. agamospermy and vegetative propagation), can be a beneficial characteristic for immigrants, and are

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common among species regarded as highly successful colonizers (Stebbins 1957, Brown and Marshall 1981). Selfing and clonally reproducing species are capable of producing progeny without the presence of a mate which, 1) frees them from the dependency of pollinators, 2) ensures reproductive success despite unfavorable conditions, 3) preserves adaptive gene complexes for all individuals within the population, and 4) allows a single individual to start an entire colony or invasion (Stebbins 1957, Williams 1975, Barrett and Richardson 1986). With clonal reproduction, progeny will be identical to their parents, and therefore, some models suggest that clonally reproducing species possess little or no genotypic variation within populations (Williams 1975). However, a second group of models claim that the level of genetic diversity in clonal species can, in fact, be similar to sexual ones (Maynard Smith 1971, Lynch 1984, Ellstrand and Roose 1987). This study examines the level and structure of genetic diversity in the clonally reproducing introduced plant species bulbous bluegrass (*Poa bulbosa* L.) (Poaceae).

Study Species

Bulbous bluegrass displays many of the ecological characteristics of a successful invasive plant species. This perennial bunchgrass is widespread throughout western Europe and the Mediterranean region (Cronquist et al. 1977), and is more rarely distributed in southern and eastern Britain (Humphries 1979). It is commonly found within pastures, roadsides, and other disturbed areas at low to middle elevations (Cronquist et al. 1977). Bulbous bluegrass was introduced in the eastern United States in 1906 (probably from Russia), and it was introduced in the western United Sates in 1915 as a contaminant in alfalfa seed (Harrison et al. 1996). Following its introduction in the West, this grass quickly spread throughout much of the Intermountain West because it is aggressive, highly competitive, persistent, and can become a dominant species in heavily disturbed (overgrazed) habitats (Harrison et al. 1996). In addition, rapid range expansion of bulbous bluegrass was facilitated by the purposeful introduction of the grass to prevent soil erosion or control more troublesome weeds (Younger and McKell 1972). Samples have been collected in New York, North Carolina, Virginia, North Dakota, Oklahoma, and most western states including California, Oregon, Washington, Idaho, Montana, Wyoming, Utah and Colorado (Hitchcock 1971).

Although bulbous bluegrass chiefly reproduces through sexual means within its native range, it is primarily an asexually reproducing species within North America where clonal production of propagules, or bulblets, have replaced the production of seeds (Hitchcock 1971, Younger and McKell 1972, Cronquist et al. 1977). Also, bulbous bluegrass possesses several bulbous bases, where each base is capable of giving rise to a new plant if detached and dispersed (Humphries 1979). Although high chromosome counts have been reported for bulbous bluegrass ($2n = 28, 35, 42$), suggesting that it is a polyploid, the literature does not indicate the type of polyploidy that occurs within this species (Cronquist et al. 1977).

Investigating the population genetics of an invading species may provide insights into the nature of its introduction into its new range. With this paper, we report results from our analysis of 10 populations of bulbous bluegrass from Idaho, Washington, and Oregon. The specific objectives of this study were to: 1) determine the level and structure of genetic variation within and among populations using enzyme electrophoresis, 2) assess the pattern of genetic variation and determine if they provide insights into introduction dynamics (single or multiple introductions events), 3) compare the results obtained for bulbous bluegrass to other studies of introduced and clonally reproducing plant species, and 4) and determine the genetic consequences of polyploidy for this species (i.e., type of polyploidy).

Materials and Methods

Plant Collections

As a first effort to describe the genetic variability of bulbous bluegrass, ten local populations were collected from various locations in Idaho, Oregon, and Washington (Figure 1, Table 1). Population collections were made between June and August, 1996, typically in disturbed habitats such as vacant lots and along roadsides. In addition, these populations also varied in their size and density (Table 1). The panicles of thirty to forty separate individuals in each population were collected at regular intervals (approximately 1 m) while walking through the population and stored within separate envelopes. Panicles were harvested when they

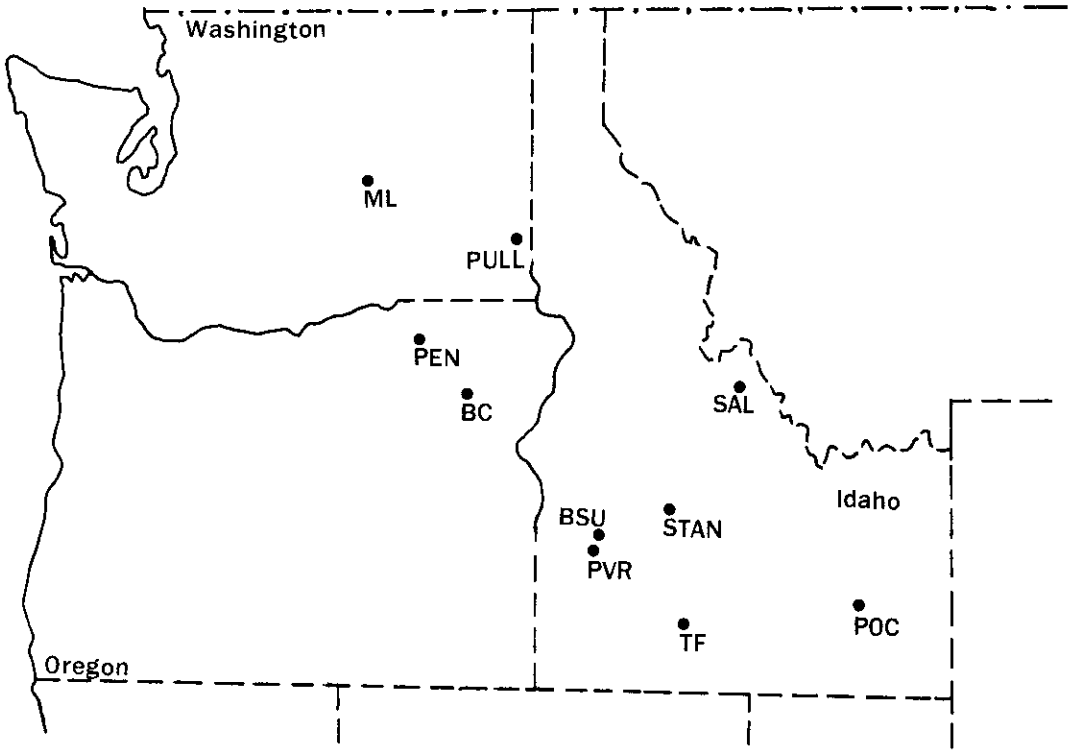

Figure 1. Map of Idaho, Oregon, and Washington, showing the locations of the 10 populations of bulbous bluegrass sampled and analyzed in this study. See Table 1 for names of sampled sites corresponding to the population abbreviations.

TABLE 1. Collection data for bulbous bluegrass population from Idaho, Oregon, and Washington, population abbreviations, and a general description of the size, density, and habitat conditions of each collection site.

State	County	Location	Population Abbreviation	Population Description
Idaho	1. Ada	Boise-Boise State University	BSU	large, high density, running path along the Boise River behind Boise State University
	2. Ada	Boise-Pleasant Valley Road	PVR	large, high density, along Pleasant Valley Road near Idaho State Penitentiary
	3. Twin Falls	Twin Falls	TF	medium, moderate density, beneath overpass
	4. Bannock	Pocatello	POC	small, moderate density, vacant lot
	5. Custer	Stanley	STAN	moderate, moderate density, dirt road on west side of Salmon River at Hell Roaring lower trailhead
	6. Lemhi	Salmon	SAL	small, low density, roadside near vacant lot
Oregon	7. Baker	Baker City	BC	large, high density, corner hillside lot
	8. Umatilla	Pendleton	PEN	moderate, high density, roadside near Eastern Oregon State Correctional Institute
Washington	9. Whitman	Pullman	PULL	moderate, moderate density, E.H. Steffen Center
	10. Grant	Moses Lake	ML	large, moderate density, disturbed sagebrush habitat

contained mature bulblets. Mature bulblets are 2 mm in length, and possess broad, shiny, dark purple or black bases with protruding foliaceous tips. One bulblet from each individual in a population was randomly selected and placed on moistened filter paper in a petri dish. To break dormancy, bulbous bluegrass required a chilling treatment consisting of incubating bulblets on moistened filter paper at 5 C for 4-5 days (Ball et al. 1995). Additional water was applied as needed to prevent desiccation of the bulblets. After chilling, petri dishes were incubated at room temperature with moderate sunlight, with bulblets usually "germinating" within 24 hours. "Seedlings" were permitted to grow for approximately one week, prior to genetic analysis.

Genetic Analysis

Enzyme electrophoresis was performed according to the methods of Soltis et al. (1983). Harvested "seedling" tissue was macerated in tris-HCL grinding buffer-PVP solution. Final concentration of PVP (MW 40,000) in the grinding buffer was 12% (w/v). Starch concentration of the gels was 12.5% (w/v). Enzyme activity for alcohol dehydrogenase (ADH), aldolase (ALD), glutamate dehydrogenase (GDH), glutamate oxalacetate transaminase (GOT), malic enzyme (ME), phosphoglucoisomerase (PGI), and triose-phosphate isomerase (TPI) were visualized using buffer system 8 (Novak et al. 1991). Enzyme activity for glyceraldehyde-3-phosphate dehydrogenase (G3PDH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), and shikimate dehydrogenase (SkDH) were visualized using buffer system 1; while enzyme activity for glucose-6-phosphate dehydrogenase (G6PDH), malate dehydrogenase (MDH), and phosphoglucomutase (PGM) were visualized using buffer system 9 (Soltis, et al. 1983). Reliable interpretation of the banding patterns of MDH and PGM was obtained by comparing results obtained from both system 1 and system 9. Enzyme staining procedures followed the methods of Soltis et al. (1983), except for ADH, which followed Haufler (1985). ALD and TPI were stained using the agarose overlay method.

Enzyme banding patterns for each individual were recorded, and genotypes were inferred based on known enzyme subunit structure and compartmentalization for diploid seed plants (Weeden and Wendel 1989). However, the complex banding patterns observed for bulbous bluegrass were not consistent with diploid gene expression, but instead were more consistent with the patterns previously reported for autotetraploid plant taxa (Soltis and Rieseberg 1986; Soltis and Soltis 1988, Soltis and Soltis 1989a, Soltis and Soltis 1989b, Wolf et al. 1989). Because of these similarities, we assumed that the populations of bulbous bluegrass analyzed in this study are autotetraploid, and assigned genotypes based on the relative staining intensity and number of enzyme bands scored at each polymorphic locus. Additionally, we reanalyzed many individuals to ensure that our assignments of genotypes, based on the criteria described above, were consistent.

Genetic diversity for all 10 populations of bulbous bluegrass was assessed using the mean number of alleles per locus (A), the percent polymorphic loci per population ($\%P$) and the observed mean heterozygosity (H_o). A locus was defined as being polymorphic if one or more individual(s) within a population possessed a genotype that differed from other individuals in that population, at that locus. Observed mean heterozygosity was determined by the direct count method. Mean values for A and $\%P$ for all ten populations of bulbous bluegrass, at both the species and population levels, were compared to the mean values of A and $\%P$ compiled by Hamrick and Godt (1989) from over 400 published studies of plant species using enzyme electrophoresis. Because an autotetraploid individual can maintain as many as three or four alleles at a single locus (due to tetrasomic inheritance), the proportion of individuals exhibiting three or more alleles at one or more locus was calculated for each population (Soltis and Soltis 1989b). In this study, clonal structure was assessed by calculating the total number of unique multilocus genotypes, as well as the distribution of multilocus genotypes within and among populations. Multilocus genotypes were assigned by examining their allelic composition at all polymorphic loci.

Results

Genetic Diversity

A total of 264 bulbous bluegrass individuals from 10 populations in Idaho, Oregon, and Washington were analyzed for genetic diversity, with an average of approximately 26 individuals per

population. All enzyme bands scored in this study migrated anodally from the origin. The 14 enzymes were coded for by 19 putative genetic loci. Across all populations nine of 19 loci (47.4%) were polymorphic: *Adh*, *G3pdh*, *Idh-1*, *Mdh-1*, *Mdh-2*, *Pgi-1*, *Pgi-2*, *Pgm-1*, and *6Pgd-1* (Table 2); all other loci were monomorphic. Although the populations of bulbous bluegrass analyzed in this study generally possessed similar patterns of allelic variability, some differences among populations were observed. For instance, *Adha* was detected in only five of 10 populations (TF, POC, BC, PEN, and ML); whereas, *Adhc* occurred in all populations except PVR, PEN, PULL, and ML. *G3pdhb* was detected in only two populations: TF and STAN. Eight of 10 populations were polymorphic at *Idh-1*, while PVR and PULL were fixed for *Idh-1a*. Only BSU, PVR, and PULL were polymorphic at *Mdh-1*, all other populations were fixed for *Mdh-1a*. With the exception of STAN (which was fixed for *Pgi-2c*) and PEN (with both *Pgi-2b* and *Pgi-2c*), all 10 populations possessed at least three alleles at *Mdh-2* and *Pgi-2*. All 10 populations were polymorphic at *Pgi-1* and *6Pgd-1*. Finally, six of 10 populations were polymorphic at *Pgm-1*, while PVR, POC, PULL, and ML were fixed for *Pgm-1a*.

A total of 34 alleles were detected at the 19 scored loci, with a mean value of 1.79 alleles/locus for the species. Averaged across all populations, the mean value for *A* was 1.54, *%P* was 27.9, and H_o was 0.202 (Table 3). The highest values for *A* and *%P* (1.74 and 36.8%, respectively) were observed in the TF population. The same value for *%P* (36.8%) was found in the BSU and PEN populations, although values for *A* in the latter two populations were slightly lower than TF. By far, the lowest levels of %P (5.3) and A (1.42) were observed in the PVR population, even though PVR also had the second highest H_o value (0.263). The highest value (H_o = 0.309) was detected in the STAN population while the lowest value of 0.124 was observed in the SAL population. Mean values for *A* and *%P* for all ten

TABLE 2. Allele frequencies at nine polymorphic loci for each population of bulbous bluegrass. Population abbreviations are given in Table 1.

		Populations									
Locus	Alleles	BSU	PVR	TF	POC	STAN	SAL	BC	PEN	PULL	ML
Adh	*a*	-	-	0.138	0.268	-	-	0.069	0.057	-	0.052
	b	0.875	1.000	0.793	0.723	0.917	0.971	0.922	0.943	1.000	0.948
	c	0.125	-	0.069	0.009	0.083	0.029	0.009	-	-	-
G3pdh	*a*	1.000	1.000	0.888	1.000	0.750	1.000	1.000	1.000	1.000	1.000
	b	-	-	0.112	-	0.250	-	-	-	-	-
Idh-1	*a*	0.644	1.000	0.517	0.036	0.833	0.118	0.198	0.179	1.000	0.042
	b	0.356	-	0.462	0.964	0.167	0.882	0.802	0.821	-	0.958
Mdh-1	*a*	0.990	0.750	1.000	1.000	1.000	1.000	1.000	1.000	0.946	1.000
	b	0.010	0.250	-	-	-	-	-	-	0.054	-
Mdh-2	*a*	0.317	0.250	0.259	0.375	0.250	0.691	0.405	0.579	0.250	0.281
	b	0.289	0.500	0.207	0.018	0.50	0.059	0.052	0.028	0.500	0.021
	c	0.077	-	0.276	0.232	-	-	0.138	0.143	-	0.281
	d	0.163	0.250	0.103	0.009	0.250	0.029	0.026	0.014	0.250	0.010
	e	0.154	-	0.155	0.366	-	0.221	0.379	0.236	-	0.219
Pgi-1	*a*	0.250	0.250	0.250	0.250	0.350	0.265	0.250	0.329	0.250	0.250
	b	0.750	0.750	0.750	0.750	0.660	0.735	0.750	0.671	0.750	0.750
Pgi-2	*a*	0.038	0.250	0.103	0.116	-	-	0.035	-	0.239	0.031
	b	0.174	0.250	0.009	0.080	0.167	-	0.017	0.186	0.294	0.031
	c	0.750	0.250	0.853	0.804	0.667	1.000	0.948	0.814	0.467	0.938
	d	0.038	0.250	0.035	-	0.167	-	-	-	-	-
Pgm-1	*a*	0.933	1.000	0.922	1.000	0.833	0.971	0.647	0.629	1.000	1.000
	b	0.067	-	0.78	-	0.167	0.029	0.353	0.371	-	-
6Pgd-1	*a*	0.577	0.607	0.638	0.955	0.667	0.912	0.888	0.829	0.641	0.958
	b	0.423	0.393	0.362	0.045	0.333	0.088	0.112	0.171	0.359	0.042

TABLE 3. Genetic variability statistics for bulbous bluegrass populations. Description of A, $\%P$, and H_o are given in the text. Proportion refers to the number of individuals possessing three or more alleles at one or more loci in these populations.

State	Population	N	A	$\%P$	H_o	Proportion	Multilocus Genotypes
Idaho	1. Boise-BSU	26	1.68	36.8	0.227	0.77	12
	2. Boise-PVR	21	1.42	5.3	0.263	1.00	2
	3. Twin Falls	29	1.74	36.8	0.243	0.97	15
	4. Pocatello	28	1.58	26.3	0.177	0.50	7
	5. Stanley	15	1.53	31.6	0.309	1.00	7
	6. Salmon	34	1.42	31.6	0.124	0.12	3
Oregon	7. Baker City	29	1.58	31.6	0.160	0.38	10
	8. Pendleton	35	1.53	36.8	0.174	0.34	24
Washington	9. Pullman	23	1.37	15.8	0.222	1.00	5
	10. Moses Lake	24	1.53	26.3	0.125	0.33	11
All populations		264	1.54	27.9	0.202	0.64	9.6

populations of bulbous bluegrass, at both the species and population levels, were similar to the mean values of A and $\%P$ compiled by Hamrick and Godt (1989) (Table 4).

The possession of loci with three or more alleles at a locus is one of the genetic consequences of autopolyploidy (Soltis and Soltis 1988, Soltis and Soltis 1989b). Therefore, autotetraploid plants exhibit "enzyme multiplicity" (i.e., they produce multiple enzyme forms, as well as more kinds of hybrid enzymes for multimeric enzymes). For the ten populations of bulbous bluegrass analyzed in this study, enzyme multiplicity was observed at *Mdh-2* and *Pgi-2*. Even though three of these 10 populations of bulbous bluegrass (TF, POC, BC) possessed three alleles at *Adh*, no one individual exhibited enzyme multiplicity at this locus. The mean proportion of individuals having three or more alleles at one or more loci for all ten populations was 0.64 (Table 3). The highest proportion (1.00) occurred within two Idaho populations (PVR and STAN) and one Washington population (PULL), while the lowest proportion (0.12) was found within the SAL population of Idaho. All ten populations possessed individuals exhibiting enzyme multiplicity at *Mdh-2* (with a minimum of four individuals in SAL), while only five of ten populations (BSU, PVR, POC, STAN, and PULL) contained individuals with enzyme multiplicity at *Pgi-2* (data not shown). Every individual in PVR exhibited enzyme multiplicity for both *Mdh-2* and *Pgi-2*.

TABLE 4. Comparison of genetic variability in bulbous bluegrass with other plant species, based on enzyme electrophoresis data. Data for the other plant species were compiled by Hamrick and Godt (1990).

	Species level	
	A	$\%P$
Monocots	2.38	59.2
Herbaceous short-lived	1.42	41.3
Temperate plants	1.91	48.5
Gravity-dispersed seeds	1.81	45.7
Asexual and sexual	1.69	43.8
Early successional	1.98	49.0
All other plants	1.96	50.5
Bulbous bluegrass	1.79	47.4
	Population level	
	A	$\%P$
Monocots	1.66	40.3
Herbaceous short-lived	1.40	28.0
Temperate plants	1.51	32.6
Gravity-dispersed seeds	1.45	29.8
Asexual and sexual	1.47	29.4
Early successional	1.46	29.6
All other plants	1.53	34.2
Bulbous bluegrass	1.54	27.9

Clonal Structure

A total of 84 multilocus genotypes were detected within the 10 populations of bulbous bluegrass analyzed in this study (Figure 2). Seventy-six multilocus genotypes (90.5%) were restricted to

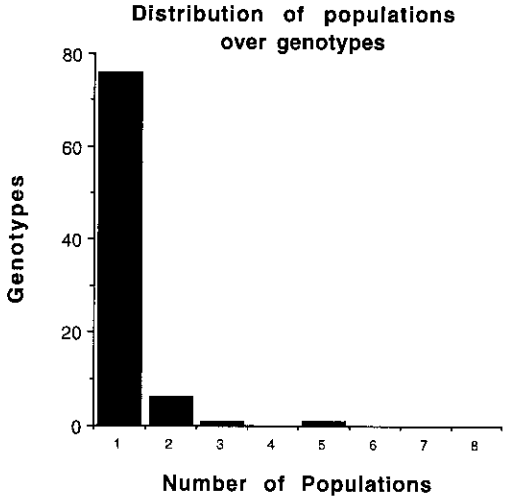

Figure 2. Histogram illustrating the distribution of 84 multilocus genotypes among the 10 populations of bulbous bluegrass. "Number of Populations" equals the number of populations in which each genotype was detected.

a single (but not the same) population. Six different multilocus genotypes were found within two populations, one genotype was found within three, and the most widespread genotype, designated #2, occurred in five of the ten populations (BSU, POC, BC, PEN, and ML). The mean number of populations that each genotype occurred in was 1.17. The fewest number of multilocus genotypes that occurred within a single population (PVR) was two, while the highest number of genotypes found within a population (PEN) was 24 (Table 3, Figure 3). On average, each population of bulbous bluegrass included in this analysis possessed 9.6 multilocus genotypes (Table 3).

Discussion

Genetic Diversity

Our analysis of these 10 populations of bulbous bluegrass from Idaho, Oregon, and Washington indicates that this species displays surprisingly high levels of genetic diversity at the species and

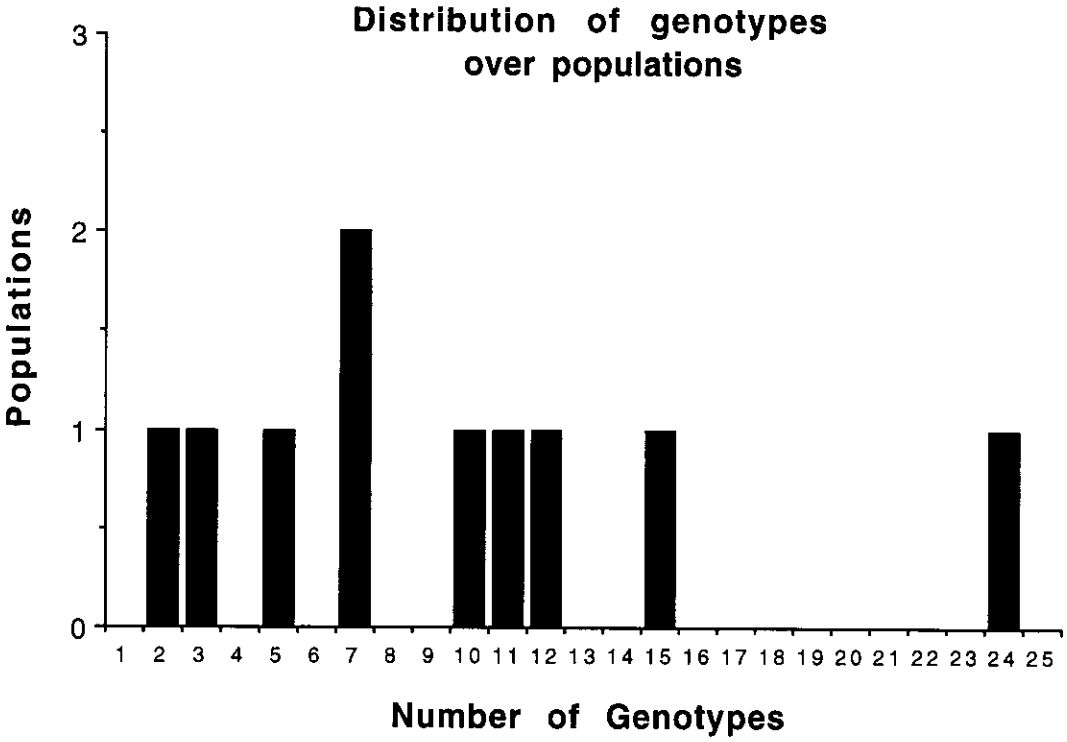

Figure 3. Histogram displaying the number of genotypes detected in each of the 10 populations of bulbous bluegrass. "Populations" equals the actual number of sites with a given number of genotypes.

population level. At the species level, the mean values for A and %P reported for bulbous bluegrass (1.79 and 47.4, respectively) are quite similar to the values reported for other plant species (1.96 and 50.5, respectively) (Table 4). At the population level, similar mean values of *A* were detected for bulbous bluegrass and other plants (1.53 and 1.54, respectively), while the mean value of *%P* for bulbous bluegrass (27.9) was somewhat lower when compared with other plants (34.2). However, if the results for PVR and PULL (%P = 5.3 and 15.8, respectively) are excluded from the analysis, the value of %P for the remaining eight populations of bulbous bluegrass (32.2) is very comparable to the value reported for other plants. These observations are surprising because most of these other species reproduce sexually, and sexual species are generally believed to possess more diversity than species that reproduce clonally. With the exception of the values for other monocots, bulbous bluegrass has similar amounts of genetic diversity compared to plants with similar life history characteristics, at both the species and population level, as summarized by Hamrick and Godt (1989): herbaceous short-lived perennials, temperate plants, plants with gravity-dispersed seeds, plants that can reproduce both sexually and asexual, and early successional plants (Table 4).

Considering that bulbous bluegrass is an introduced plant species that reproduces by clonal means, the findings of this study are made even more surprising. Low levels of genetic diversity have often been reported within and among populations of introduced plant species (Brown and Marshall 1981, Barrett and Richardson 1986, Barrett and Shore 1989), especially among introduced plants that reproduce clonally (Barrett and Richardson 1986, Barrett and Shore 1989). For instance, the level of genetic variation detected in these 10 populations of bulbous bluegrass was higher than that reported for introduced plants that reproduce sexually such as Noogoora burr (*Xanthium strumarium*—Moran and Marshall 1978), little jack (*Emex spinosa*—Marshall and Weiss 1982), witchweed (*Striga asiatica*—Werth et. al. 1984), Johnson grass (*Sorghum halepense*—Warwick et. al. 1984), and cheatgrass (*Bromus tectorum*—Novak et. al. 1991, Novak and Mack 1993). In addition, the level of genetic variation in bulbous bluegrass was substantially higher than the level reported for most introduced plants that exhibit clonal reproduction such as skeleton weed (*Chondrilla juncea*—Burdon et. al. 1980), dandelion (*Taraxacum officinale*—Lyman and Ellstrand 1984), and white bryony (*Bryonia alba*—Novak and Mack 1995).

Bulbous bluegrass also possesses high levels of heterozygosity (H_0 = 0.202), and 64% of all individuals, on average, maintained as many as three or more alleles at one or more loci (Table 3). Our findings are consistent with previous reports describing the genetic consequences of autopolyploidy: autopolyploid plants frequently possess higher levels of heterozygosity, because of enzyme multiplicity, when compared to diploid plants (Soltis and Rieseberg 1986; Soltis and Soltis 1988, Soltis and Soltis 1989a, Soltis and Soltis 1989b, Wolf et al. 1989). Based on the results of this study, and the high chromosome counts commonly reported for bulbous bluegrass, it is likely that this species is an autopolyploid. This likelihood probably explains the higher levels of genetic variation reported for bulbous bluegrass when compared to the other introduced plant species listed above, which are primarily diploids. Further insights into the type of autopolyploidy exhibited by bulbous bluegrass will be obtained by performing chromosomes counts on the populations analyzed in this study.

Clonal Structure

Insights into the clonal structure of bulbous bluegrass are provided by examining the number and distribution of multilocus genotypes. The distribution of multilocus genotypes within and among populations of this grass is in general agreement with results reported for other clonal plant species (for a review see Ellstrand and Roose 1987). The vast majority of genotypes (76 of 84) possessed a local distribution, being restricted to just a single (although not the same) population, and the most widespread multilocus genotype (#2) was detected in only five of 10 populations (BSU, POC, BC, PEN, and ML). In addition, none of the multilocus genotypes detected in multiple populations appeared to have a strong geographical pattern to their distribution. However, several of these genotypes were consistently detected in either the BSU, POC, BC, or ML populations, with three of these populations (BSU, BC, and ML) located in western portions of the sampled range. For instance, genotype #4 was detected in BSU and ML, genotype #7 was detected in BSU and BC, and genotype #19 was detected in BC and ML (data not shown). The abundance and distribution of these multilocus genotypes may have resulted from multiple introductions of this grass into the area

sampled and its subsequent accidental spread. Alternatively, this abundance and distribution may have resulted from multiple introductions and subsequent purposeful introduction of the grass to prevent soil erosion or control of more troublesome weeds. Purposeful introductions are a more likely means of spread because bulbous bluegrass bulblets appear to possess limited dispersal capabilities.

Bulbous bluegrass did not have a single population that was uniclonal (Table 3). The fewest number of genotypes detected within a single population (PVR) was two, while 24 different genotypes were detected in the PEN population. These 24 distinct genotypes were detected from a total of 36 individuals, indicating a high level of diversity within this population. These findings are in general agreement with the results reported for other clonal plant species. In their analysis of 27 studies, Ellstrand and Roose (1987) reported that the percentage of multiclonal populations per study tended to be high, with a mean of 77% and a range of 0-100%. The results obtained for bulbous bluegrass are clearly at the high end of this range. Ellstrand and Roose (1987) also reported that the average number of genotypes detected per population was 16.1. However, this value is inflated because one of these species possessed 167 genotypes per population, and if this value is eliminated from their calculation the average number of genotypes per population is reduced to 10.8 The number of genotypes detected per population for bulbous bluegrass is similar to the value reported above: on average, each population possessed 9.6 multilocus genotypes (Table 3).

The number of multilocus genotypes that bulbous bluegrass possesses is remarkably high for an introduced clonal species. Theory predicts that the number of genotypes detected is positively correlated with the number of individuals analyzed per population, and the number of characters (loci) employed (Ellstrand and Roose 1987, Bayer 1990). The large number of multilocus genotypes (clones) detected in this study may be due to the fact that we analyzed, on average, 26 individuals per population, and scored variability in these populations using the results of nine polymorphic loci. A slightly higher number of characters were employed for the identification of clones in the studies summarized by Ellstrand and Roose (1987): on average, 11 characters were used.

In addition, such high levels of genetic variation for this species may also be due to several other factors: 1) multiple introduction of different genotypes may have occurred directly into these locations, and/or 2) some sexual reproduction may have occurred within various locations in its introduced range even though all of the reproductive structures analyzed from these populations were bulblets.

Conclusions

Bulbous bluegrass possesses a large amount of genetic diversity despite the widely held belief that clonal species do not possess as much diversity as sexual ones. Therefore, our results are in general agreement with the second group of models described above, and suggest that clonal species are capable of possessing as much genetic diversity as sexual species (Maynard Smith 1971, Lynch 1984, Ellstrand and Roose 1987). Multiple introduction events are a likely cause of the high level of genetic diversity within populations and the restricted distribution of multilocus genotypes among populations. These results join a growing body of genetic evidence that suggests multiple introductions of an alien species may be more common than previously thought. In addition, a small amount of sexual reproduction may also have contributed to the high level of genetic diversity observed for this species. Finally, it is interesting to note that the role of introduction events, sexual reproduction, and autopolyploidy in off-setting the anticipated loss of genetic variation in these populations can only be fully resolved by the analysis of populations from the native range. As this project expands, it should also include the analysis of Eurasian populations of bulbous bluegrass.

Acknowledgements

This study was conducted in partial fulfillment of the requirements for the undergraduate Honors Program at Boise State University by AYW. We thank Jim Smith, Loren Carter, Regina Rochefort, Carla Stern, and an anonymous reviewer for constructive comments on the manuscript; Jennifer Ott for assistance in the laboratory; and Kam Spelman for assistance in the preparation on tables. This project was funded by a grant from the Faculty Research Program at Boise State Unversity to SJN, and was aided by a Grant-in-Aid of Research from Sigma Xi, the Scientific Research Society, to AYW.

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Received 18 June 1997
Accepted for publication 11 September 1997