Theor Appl Genet (2000) 101:70–79

L.W. Mengistu · G.W. Mueller-Warrant · R.E. Barker

Genetic diversity of *Poa annua* in western Oregon grass seed crops

Received: 24 July 1999 / 11 November 1999

Abstract The genetic diversity of *Poa annua* L. populations collected from western Oregon grass-seed fields was surveyed using 18 randomly amplified polymorphic DNA **(**RAPD) markers. Markers from 1357 individual plants from 47 populations collected at three sampling dates (fall, winter, and spring) for 16 sites were used to measure genetic diversity within and among populations. Site histories varied from low to high herbicide selection pressure, and some sites were subdivided by 3 years of differing post-harvest residue management. Gene diversity statistics, simple frequency of haplotype occurrence, and analysis of molecular variance (AMOVA) revealed the presence of significant variability in *P. annua* among sites, among collection dates within sites, and within collection dates. Nei gene-diversity statistics and population-differentiation parameters indicated that *P. annua* populations were highly diverse. Mean Nei gene diversity (h) for all 47 populations was 0.241 and total diversity (H_T) was 0.245. A greater proportion of this diversity, however, was within (H_S=0.209) rather than among (G_{ST}=0.146) populations. When populations were grouped by season of collection, within-group diversity was H_S=0.241, while among-group diversity was G_{ST}=0.017. When populations were grouped by site, within-group diversity was H_S=0.224, while among-group diversity was G_{ST}=0.087. The diversity among populations within season for fall, winter, and spring collections was G_{ST}=0.121, 0.142, and 0.133, respectively. Populations collected from fields with histories of high herbicide selection pressure showed low differentiation among collection dates, with G_{ST} as low as 0.016, whereas those collected from fields with low herbicide selection pressure showed greater differentiation among collection dates, with G_{ST} as high as 0.125. At high selection-pressure sites, populations were also lower in gene diversity (as low as h=0.155), while at low selection-pressure sites there was higher gene diversity (as high as h=0.286). The site to site variability was greater for the high selection-pressure sites (G_{ST}=0.107 or 69% of the total among-population variance), while the season of germination variability was greater at sites of low herbicide-selection pressure (G_{ST}=0.067, or 70% of the total among-population variance). High initial diversity coupled with a long-term re-supply of genotypes from the seed bank must have been factors in maintaining the genetic diversity of this weed despite the intensive use of herbicides. Knowledge of the genetic diversity of Willamette Valley *P. annua* should help in formulating more effective strategies for managing this weed.

Key words *Poa annua* L. · Genetic diversity · RAPD · Turfgrass weeds · Selection pressure · Analysis of molecular variance · AMOVA · POPGENE

Communicated by A.L. Kahler

L.W. Mengistu
Crop and Soil Science Department, Oregon State University, Corvallis, OR 97330, USA

G.W. Mueller-Warrant (✉) · R.E. Barker
USDA-ARS, National Forage Seed Production Research Center, 3450 SW Campus Way, Corvallis, OR 97331-7102, USA
e-mail: muellerg@ucs.orst.edu
Fax: +1-541-750 8750

Present address:
L.W. Mengistu
Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA

Introduction

Poa annua is a common weed wherever cool-season turfgrasses are grown. Its presence as a contaminant in grass seed crops is one of the most serious weed problems facing grass seed growers. Recent reductions in field burning in the Pacific Northwest USA and cancellations of registrations for older herbicides have created increased reliance on the several remaining herbicides registered for *P. annua* control in grass seed crops. Not unexpectedly, biotypes of *P. annua* resistant to two widely used herbicides, diuron [*N*'-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea] and ethofumesate [(±)-2-ethoxy-2,3-dihy-

dro-3,3-dimethyl-5-benzofuranyl methanesulfonate], have now become serious threats to the continued production of *Poa*-free grass seed (Heap 1995). Cool-season grass seed production and reports of herbicide-resistant *P. annua* are both concentrated in the Willamette Valley of western Oregon.

Two life history forms of *P. annua* are well known, an annual subtype that flowers quickly and a perennial subtype that is slower to flower and may live for several years (Tutin 1957; Holm et al. 1997). Evidence for the existence of both of these types in combination has been documented in various surveys (Warwick and Briggs 1978; Warwick 1979). However, differentiation of populations in response to the environment has also been seen (Gibeault and Goetze 1973; Warwick 1979). *P. annua* shows great diversity in vernalization requirements, from none for annuals to more exacting requirements for perennials (Johnson and White 1997). Within perennials, vernalization needs vary from population to population. Seed dormancy studies showed rapid differentiation in temperature-enforced dormancy among populations (Wu et al. 1987). Genetic variation within and among populations of *P. annua* has generally been significant (Ellis et al. 1970). Sweeney and Danneberger (1995) used RAPDs to characterize *P. annua* populations in adjacent golf course greens and fairways. They found genetic differences among some of the populations, implying that gene flow was limited. Other studies using isozymes indicated outcrossing in *P. annua* was 10–16% (Darmency and Gasquez 1981), and up to 22% if plant density was high (Darmency et al. 1992).

Methods based on arbitrarily primed-PCR (Welsh and McClelland 1990) and RAPDs (Williams et al. 1990) have become widely used tools for studying genetic variation in natural populations. The RAPD technique and AMOVA (Excoffier et al. 1992) were used in outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.] populations to partition genetic variation within populations, among populations, and among regions (Huff et al. 1993). RAPDs are predominantly dominant markers, and it is difficult to distinguish homozygotic from heterozygotic RAPD phenotypes in diploid tissue. The program POPGENE Version 1.21 (Yeh et al. 1997) was recently released to analyze RAPDs and other data sets in structured populations. This software allows the user to specify whether the populations are in Hardy-Weinberg equilibrium or disequilibrium, whether a marker is dominant or co-dominant, and allows inclusion of an inbreeding coefficient (F_{IS}) from known co-dominant markers where there is a departure from Hardy-Weinberg equilibrium. POPGENE computes both comprehensive genetic statistics, e.g., RAPD frequencies using an algorithm by Chong et al. (1994), gene diversity, genetic distance, G-statistics, F-statistics, and more complex genetic statistics such as gene flow, neutrality tests, linkage disequilibria, and multi-locus structure (Yeh et al. 1997). Studies of genetic variation in plants have typically used Nei's genetic diversity statistics or Wright's F-statistics as tools for describing the extent of differentiation among populations (Wright 1969; Nei 1973; Hamrick and Godt 1989).

In our study, genetic diversity was assayed using RAPD markers on individual seedlings collected in a structured design. We conducted this survey of genetic diversity of *P. annua* in western Oregon to learn more about the evolution of this weed. Our long-range goal was to modify old and develop new seed production and weed control practices that will prolong our ability to control this species by delaying the advent and minimizing the extent of resistance to new herbicides and other management practices.

Materials and methods

Plant collection

A total of 1578 two-leaf stage *P. annua* seedlings were collected from ten grass seed production fields in the Willamette Valley of Oregon from early fall of 1994 through early spring of 1995 (Table 1). Two of the ten fields were further subdivided on the basis of post-harvest residue management treatments imposed annually since 1992, creating a total of 16 sampling sites. Permanent plots were marked in the fields, and young seedlings were collected from within the same 10-m^2 area at each site in all three collection periods (early fall 1994, early winter 1994–95, and early spring 1995). Seedlings were transferred to the greenhouse and grown in 150-ml plastic cone-shaped pots until fresh leaves were harvested for DNA extraction. DNA from each sample was extracted using a rapid one-step extraction method (ROSE) as described by Stiener et al. (1995), and kept at –20°C until used for PCR.

PCR conditions and DNA amplifications

PCR was performed in a 12-µl reaction vol under conditions optimized for our extraction of *P. annua* leaf DNA. Using micropipettes, 8.4 µl of reaction mix [1×Stoffel buffer, 3.75 mM $MgCl_2$, 50 µM of each dNTP, 0.5 pmol of primer (Operon B-08 primer, sequence 5´GTCCACACGG 3´), and 1 unit of *Taq* Stoffel fragment] was first aliquotted into 96-well assay plates and then overlaid with three drops of mineral oil. Finally, 3.6 µl of the diluted DNA samples was added to the reaction mixture under the oil. The thermal cycling program started with denaturation for 7 min at 94°C followed by 43 cycles of 30 s denaturation at 94°C, 1 min annealing at 46°C, a 1°C per 3-sec ramp, and extension at 72°C for 2 min. A final extension at 72°C for 5 min concluded the DNA amplification.

Electrophoresis and silver staining

Amplification products were separated by polyacrylamide gel-electrophoresis and stained with silver. Gels were made from Tris-HCl (pH 8.8), acrylamide (7.5% final), ammonium persulfate, and TEMED (N,N,N´,N´-tetramethylethylenediamine). Following polymerization of the gel, wells were loaded with 6 µl of each amplification product mixed with 9 µl of loading buffer. Three standard markers were included in every gel, and gels were run at 180 V for 45 min. The staining protocol included incubation with silver nitrate solution (50 ml per gel) for 10 min., developing with sodium carbonate solution (50 ml per gel), and fixing with 7.5% acetic acid (Bassam et al. 1991). The gels were dried between sheets of gel-drying film (Promega) and scored by hand for presence or absence of 18 clearly distinguishable, repeatable bands.

Table 1 *Poa annua* accessions collected from grass seed fields of western Oregon

Site	Number of samples collected[a]	Current crop	Brief site history
Bowers farm	540 samples in fall, winter, and spring (90 samples per treatment for 6 residue treatments)	Perennial ryegrass	Replicated field trial with six residue management treatments imposed for 3 years prior to sampling
Glaser meadowfoam	84 samples in fall and winter only. No *P. annua* available for collection in spring	Meadowfoam	Rotated to meadowfoam because of high density of resistant *P. annua*
Glaser perennial ryegrass	126 samples in fall, winter, and spring	Perennial ryegrass	Long-term grass seed production, resistant *P. annua* present
Pugh perennial ryegrass	126 samples in fall, winter, and spring	Perennial ryegrass	Long-term grass seed production, resistant *P. annua* present
Pugh tall fescue	126 samples in fall, winter, and spring	Tall fescue	Long-term grass seed production, resistant *P. annua* present
Hyslop Crop Science Field Laboratory	72 samples in fall, winter and spring (36 samples per treatment for two residue treatments)	Perennial ryegrass	Replicated field trial with two residue management treatments imposed for 3 years prior to sampling
McLagan farm, Bell Plain Rd.	126 samples in fall, winter, and spring	Perennial ryegrass	Long-term grass seed production, resistant *P. annua* present
McLagan farm, Pugh Rd.	126 samples in fall, winter, and spring	Perennial ryegrass	Long-term grass seed production, resistant *P. annua* present
Coon bale/flail/rake	126 samples in fall, winter, and spring	Perennial ryegrass	Long-term grass seed production, resistant *P. annua* present
Coon full straw chop	126 samples in fall, winter, and spring	Perennial ryegrass	Long-term grass seed production, resistant *P. annua* present

[a] A total of 1578 samples were collected from all sites

Data analysis

Eighteen low-molecular-weight RAPD bands (loci) ranging from 230 to 872 bp from 1357 out of the original 1578 samples were selected for analysis. Only bands that were clearly distinguishable and repeatable between duplicate samples were used. The 221 samples that amplified poorly and could not be reliably scored for some or all of the 18 selected bands were dropped from further analysis. The basic data structure consisted of a binomial (0, 1) matrix of 1357 rows, with one column identifying the individual and 18 columns describing the presence or absence of each of the 18 bands.

POPGENE version 1.21 was used to analyze gene diversity and population differentiation. To do this, first the RAPD data from the 1357 individual plants were grouped into 47 populations based on the site and time of collection. Then the 47 populations were regrouped based on the three germination periods or on the 16 sites. Additional groupings were performed into those only from the Bowers farm (which were mostly diuron-susceptible types) and those not from Bowers (which were mostly diuron-resistant types). POPGENE was convenient for analysis of our data because we were able to specify the populations to be in Hardy-Weinberg disequilibrium. We added an inbreeding coefficient of F_{IS}=0.64 in all the POPGENE analyses, assuming that the species has 22% outcrossing as suggested by Darmency et al. (1992).

Data were also statistically analyzed using ARLEQUIN, the updated version of AMOVA (Excoffier et al. 1992; Huff et al. 1993), on the Euclidean square distances between haplotypes. Our general structure for ARLEQUIN was sites, collection dates within sites, and within-collection dates. We also performed a non-random subdivision of our population into the most commonly occurring types (those present as more than one individual across all sites) and the unique types. There were 1045 individuals with the 184 most common haplotypes, and 312 unique individuals.

NTSYS-pc (Rohlf 1993) was used to plot data in several formats, but only the unweighted pair group method with arithmetic averaging (UPGMA) trees is shown. Dendrograms were based on Nei's (1978) genetic distance between the populations using UPGMA modified from the NEIGHBOR procedure of PHYLIP version 3.5.

Results

Genetic diversity of 47 populations analyzed as a single group

For the 18 RAPD bands analyzed, populations varied in the number and percent of polymorphic loci, ranging from 8 out of 18 (44%, Hyslop bale/flail/rake winter collection) to 16 out of 18 (89%, Bowers full straw chop fall and spring collections). The observed number of alleles (n_a) per population varied from 1.44 to 1.89 (Table 2). The mean effective number of alleles (Kimura and Crow 1964; Hartl and Clark 1989) for all 47 populations was n_e=1.40, with a range from 1.19 to 1.48. Mean Nei gene diversity for the 47 populations was h=0.241, with diversity of the populations varying from h=0.119 (Coon full straw winter collection) to h=0.293 (Bowers bale/flail/rake fall collection). Total diversity was H_T=0.245, diversity within populations was H_S=0.209, and diversity among populations was G_{ST}=0.146. Populations from Bowers farm had the largest values of n_e and h, while the Coon full straw chop population had the lowest. Using Slatkin and Barton's (1989) formula for estimated gene flow, N_m=0.25*(1–G_{ST})/G_{ST}, the gene

Table 2 Nei gene-diversity statistics across all 47 populations of *P. annua* from western Oregon grass seed fields based on POPGENE analysis of 18 RAPD bands. F, W, S=fall, winter and spring collections; n_a=Observed number of alleles; n_e=Effective number of alleles; h=Nei's gene diversity; H_T=Total diversity; H_S=Diversity within populations; G_{ST}=Diversity among populations. N_m=Estimated gene flow based on G_{ST}. Standard deviations of the mean are shown in parentheses

ID	Population	Sample size	Mean n_a	Mean n_e	h
1	Bowers, bale-F	27	1.78	1.40	0.250
2	Bowers, bale-W	21	1.67	1.36	0.221
3	Bowers, bale-S	27	1.67	1.32	0.191
4	Bowers, bale/flail-F	28	1.83	1.43	0.262
5	Bowers, bale/flail-W	23	1.83	1.44	0.259
6	Bowers, bale/flail-S	26	1.72	1.32	0.197
7	Bowers, bale/flail/rake-F	28	1.83	1.48	0.293
8	Bowers, bale/flail/rake-W	20	1.72	1.47	0.272
9	Bowers, bale/flail/rake-S	28	1.83	1.40	0.244
10	Bowers, bale/propane-F	29	1.67	1.40	0.239
11	Bowers, bale/propane-W	25	1.67	1.42	0.243
12	Bowers, bale/propane-S	26	1.67	1.28	0.179
13	Bowers, bale/vacuum sweep-F	30	1.78	1.39	0.234
14	Bowers, bale/vacuum sweep-W	21	1.72	1.46	0.264
15	Bowers, bale/vacuum sweep-S	25	1.72	1.36	0.220
16	Bowers, full straw-F	27	1.89	1.45	0.280
17	Bowers, full straw-W	19	1.67	1.39	0.238
18	Bowers, full straw-S	20	1.89	1.43	0.256
19	Glaser meadowfoam-F	40	1.56	1.21	0.136
20	Glaser meadowfoam-W	40	1.72	1.27	0.169
21	Glaser perennial ryegrass-F	40	1.67	1.33	0.195
22	Glaser perennial ryegrass-W	35	1.72	1.32	0.195
23	Glaser perennial ryegrass-S	39	1.78	1.32	0.190
24	Pugh perennial ryegrass-F	31	1.67	1.36	0.219
25	Pugh perennial ryegrass-W	32	1.67	1.29	0.178
26	Pugh perennial ryegrass-S	34	1.61	1.29	0.173
27	Pugh tall fescue-F	36	1.83	1.39	0.244
28	Pugh tall fescue-W	40	1.67	1.25	0.161
29	Pugh tall fescue-S	34	1.83	1.32	0.199
30	Hyslop, bale/flail/rake-F	10	1.67	1.36	0.216
31	Hyslop, bale/flail/rake-W	8	1.44	1.22	0.143
32	Hyslop, bale/flail/rake-S	10	1.67	1.31	0.197
33	Hyslop, full straw-F	10	1.56	1.32	0.191
34	Hyslop, full straw-W	8	1.67	1.36	0.221
35	Hyslop, full straw-S	10	1.61	1.29	0.187
36	McLagan Bell Plain Rd.-F	42	1.78	1.29	0.190
37	McLagan Bell Plain Rd.-W	37	1.78	1.37	0.231
38	McLagan Bell Plain Rd.-S	41	1.72	1.32	0.188
39	McLagan Pugh Rd.-F	42	1.72	1.31	0.187
40	McLagan Pugh Rd.-W	36	1.83	1.44	0.247
41	McLagan Pugh Rd.-S	41	1.72	1.35	0.205
42	Coon, bale/flail/rake-F	35	1.72	1.37	0.215
43	Coon, bale/flail/rake-W	40	1.61	1.25	0.160
44	Coon, bale/flail/rake-S	33	1.67	1.27	0.173
45	Coon, full straw-F	33	1.72	1.27	0.174
46	Coon, full straw-W	34	1.50	1.19	0.119
47	Coon, full straw-S	36	1.78	1.30	0.188
All populations		1357	2.00 (0.00)	1.40 (0.37)	0.241 (0.185)
				H_T=0.245 (0.034)	
				H_S=0.209 (0.023)	
				G_{ST}=0.146	
				$N_m(G_{ST})$=1.46	

flow between all populations was N_m=1.46. According to those authors, gene-flow values greater than one are "strong enough to prevent substantial differentiation due to genetic drift." This finding that *P. annua* populations appear to exchange genes is not too surprising, given the movement of equipment, seedstock, animals, water, and pollen between grass seed farms.

Genetic diversity and population differentiation when structured by season of collection

For further analysis with POPGENE, the same 47 populations of *P. annua* were grouped into fall, winter, and spring collections consisting of 16, 16, and 15 populations with sample sizes of 488, 439, and 430 individuals, respectively. The observed number of alleles, the effective number of alleles, Nei gene diversity, and total diversity were similar for all three collection dates (Ta-

Table 3 Nei gene diversity statistics and population differentiation parameters for three groups based on season of germination across all 47 populations. n_a=observed number of allele; n_e=effective number of alleles; h=Nei's gene diversity; H_T=total diversity; H_S=diversity within sites within each collection date; $H_{S\ all}$=diversity within all collection dates; G_{ST}=diversity among sites within each collection date; $G_{STamong}$=diversity among collection dates; G_{CS}=diversity among sites within all collection dates; N_m=gene flow based on G_{ST} or G_{CS}. Standard deviations of the means are shown in parentheses

Season of collection	Number of populations	Sample size	Mean n_a	Mean n_e	Mean h	H_T	H_S	G_{ST}	N_m
Fall	16	488	2.0 (0.0)	1.41 (0.36)	0.248 (0.175)	0.250 (0.030)	0.220 (0.022)	0.121	1.82
Winter	16	439	1.9 (0.2)	1.40 (0.38)	0.236 (0.192)	0.242 (0.038)	0.206 (0.026)	0.142	1.51
Spring	15	430	1.9 (0.2)	1.37 (0.35)	0.225 (0.183)	0.230 (0.034)	0.199 (0.024)	0.133	1.64
							$H_{S\ all}$=0.241 (0.033) $G_{STamong}$=0.017 G_{CS}=0.132 $N_m(G_{ST})$=14.2 $N_m(G_{CS})$=1.65		

Table 4 Nei gene-diversity statistics and population-differentiation parameters for 16 groups based on the site of collection across all 47 populations. n_a=observed number of allele; n_e=effective number of alleles; h=Nei's gene diversity; H_T=total diversity; H_S=diversity within collection dates within each site; $H_{S\ all}$=diversity within all sites; G_{ST}=diversity among collection dates within each site; $G_{STamong}$=diversity among sites; G_{CS}=diversity among collection dates within all sites; N_m=gene flow based on G_{ST} or G_{CS}. Standard deviations of the means are shown in parentheses

Site and residue treatment, if applicable	No. of populations	Sample size	n_a	N_e	h	H_T	H_S	G_{ST}	N_m	N_m ratio[a]
Bowers, bale	3	75	1.89	1.42	0.254	0.252	0.221	0.125	1.53	0.10
Bowers, bale/flail	3	77	1.94	1.44	0.264	0.264	0.239	0.092	2.45	0.16
Bowers, bale/flail/rake	3	76	1.94	1.48	0.286	0.286	0.269	0.058	4.05	0.27
Bowers, bale/propane	3	80	1.89	1.40	0.240	0.240	0.220	0.083	2.75	0.18
Bowers, bale/vacuum sweep	3	76	1.89	1.44	0.263	0.263	0.240	0.090	2.55	0.17
Bowers, full straw	3	66	1.94	1.47	0.281	0.278	0.258	0.072	3.2	0.21
Glaser meadowfoam	2	80	1.72	1.24	0.155	0.155	0.151	0.021	11.85	0.78
Glaser perennial ryegrass	3	114	1.89	1.33	0.198	0.198	0.194	0.025	9.8	0.64
Pugh perennial ryegrass	3	97	1.78	1.31	0.192	0.193	0.190	0.016	15.2	1.00
Pugh tall fescue	3	110	1.89	1.32	0.208	0.209	0.201	0.040	6.1	0.40
Hyslop, bale/flail/rake	3	28	1.72	1.35	0.211	0.209	0.185	0.113	1.95	0.13
Hyslop, full straw	3	28	1.72	1.33	0.213	0.215	0.200	0.069	3.35	0.22
McLagan Bell Plain Rd.	3	120	1.78	1.34	0.213	0.214	0.203	0.051	4.65	0.31
McLagan Pugh Rd.	3	119	1.89	1.37	0.221	0.223	0.213	0.044	5.45	0.36
Coon, bale/flail/rake	3	108	1.83	1.31	0.191	0.192	0.183	0.048	4.95	0.33
Coon, full straw	3	103	1.78	1.26	0.166	0.165	0.160	0.030	8.1	0.53
							$H_{S\ all}$=0.224 (0.027) $G_{STamong}$=0.087 G_{CS}=0.065 $N_m(G_{ST})$=2.62 $N_m(G_{CS})$=3.55			

[a] N_m ratio=ratio of the N_m of each site to the maximum N_m at Pugh perennial ryegrass

ble 3). The diversity among sites within fall, winter, and spring collections was G_{ST}=0.121, 0.142, and 0.133, respectively. Corresponding gene-flow estimates among sites within fall, winter, and spring collections were N_m=1.82, 1.51 and 1.64. Diversity within sites for the seasons of collection ranged from H_S=0.199 for spring to H_S=0.220 for fall. Mean diversity among sites within seasons was G_{CS}=0.132, while diversity among the three seasons was only G_{ST}=0.017 (Table 3). Estimated gene flow among the three seasons of collection was N_m=14.2, much higher than the values among sites, implying that season of collection was a much weaker barrier to genetic exchange than site of collection.

Genetic diversity and population differentiation when structured by site of collection

As we had done for the three seasons, we also structured the 47 populations into 16 groups based on the site of collection. Fifteen sites consisted of three populations each, with sample sizes ranging from 28 to 120 (Table 4). The

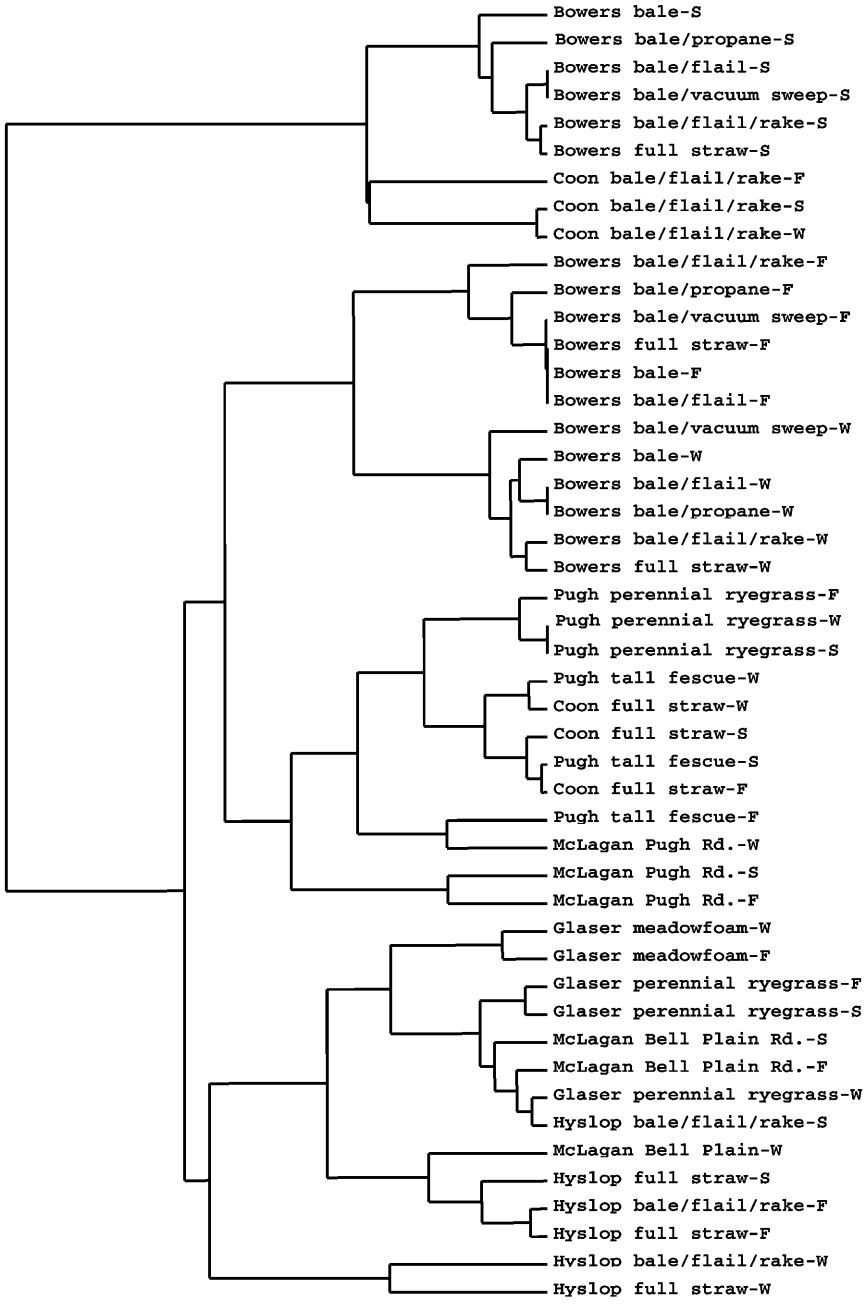

Fig. 1 Dendrogram based on Nei's genetic distance for the UPGMA method modified from the NEIGHBOR procedure of PHYLIP Version 3.5. Eighteen RAPD loci of 1357 individuals were grouped as 47 populations for analysis with POPGENE. Distance metrics among populations were based on Nei's unbiased measures of genetic identity and genetic distance

Glaser meadowfoam site consisted of only two populations, fall and winter, because the grower's application of clethodim {[(*E*,*E*)-(±)-2-[1-[(3-chloro-2-propenyl)oxy] imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} apparently controlled all the spring-germinating *P. annua*. The total diversity varied from H_T=0.155 at Glaser meadowfoam to H_T=0.286 at Bowers bale and bale/flail/rake populations. The within-site diversity over all populations was H_S=0.224, varying from H_S=0.151 at Glaser meadowfoam to H_S=0.269 at Bowers bale/flail/rake. The among collection-dates diversity within sites varied from G_{ST}=0.016 at George Pugh perennial ryegrass to G_{ST}=0.125 at Bowers bale and G_{ST}=0.113 at Hyslop bale/flail/rake. Mean genetic diversity among collection-dates within sites was G_{CS}= 0.065. The gene flow estimate among sites was N_m= 2.62, while the gene flow among collection-dates within all sites was N_m=3.55. Gene flow among collection-dates within sites varied from N_m=1.53 at Bowers bale to N_m=15.2 at Pugh perennial ryegrass.

The topology of dendrograms from the POPGENE analysis of 47 populations indicated that, although the *P. annua* populations were clearly diverse, there were obvious effects of site and collection date (Fig. 1). For example, the six residue management treatments of the 18 Bowers populations perfectly clustered into groups based on the time of germination, with the six-fall, the six-winter, and the six-spring populations clustering to-

Table 5 Summary of among- and within-group diversity for alternative grouping schemes

Grouping schemes	Total number of populations	Sample size	Mean G_{ST} among	Mean G_{CS} within	Diversity among as % of total	Diversity within as % of total
Single group of all populations	47	1357	0.146	–	100.0	–
Grouped by three seasons	47	1357	0.017	0.132	11.4	88.6
Grouped by 16 sites	47	1357	0.087	0.065	57.2	42.8
Only Bowers farm, grouped by three seasons	18	450	0.0667	0.0283	70.2	29.8
Only Bowers farm, grouped by six residue management treatments	18	450	0.0080	0.0858	8.5	91.5
Ten sites other than Bowers, grouped by three seasons	29	907	0.0059	0.1446	3.9	96.1
Ten sites other than Bowers, grouped by site	29	907	0.1070	0.0478	69.1	30.9

gether to form three distinct groups. At the Bowers site, time of germination was clearly a major factor differentiating populations. Other cluster groups with consistency in time of germination were found in Hyslop populations where the pattern of clustering was based on time of germination for fall and winter cohorts, but not spring. Examination of trees for samples collected from the other individual fields suggested the presence of small effects of collection dates within site. A substantial amount of diversity was also observed due to the site of collection. The six treatments at Bowers, which had clustered together based on time of germination, were separated from the ten remaining sites except for Coon bale/flail/rake. Populations at the remaining nine sites generally clustered based on the site of collection, with only a few populations clustering outside of their respective sites (Fig. 1). Grouping the 47 populations into 16 sites resulted in G_{ST}=57.2% among sites, with 42.8% of the variability within site (Table 5). The G_{ST} and G_{CS} values for the total 47 populations grouped into three germination seasons partitioned into G_{ST}=0.017 and G_{CS}=0.132. This means that the diversity among the three germination periods was only 11.4%, with 88.6% of the variability within season.

Comparisons of genetic diversity from low and from high herbicide selection pressure sites

An interesting result in partitioning the G_{ST} was obtained when we analyzed the six Bowers residue management populations (mostly diuron-susceptible) separately from populations at the other ten sites (all but Hyslop mostly diuron-resistant). Diversity among the six residue management treatments at Bowers was only 8.5% of the variance while the within-treatment portion was 91.5% (Table 5). When these same populations were grouped by germination periods, the among-season and within-season variance partitioned into G_{ST}=70.2% and G_{CS}=29.8%. This again indicates that a large amount of the diversity in *P. annua* populations at Bowers was attributed to the time of germination. This large effect of the time of germination was not found at the other ten sites, where the among-collection dates proportion was only 3.9% of the total variance. However, the among-sites component at the other ten sites was elevated, reaching values of G_{ST}=0.107 and G_{CS}=0.0478, indicating that nearly 69% of the variation was among sites while the remaining 31% of diversity was within site (Table 5).

Genetic diversity and hierarchical analysis of molecular variance

Analysis of molecular variance conducted over all 16 sites revealed the presence of highly significant effects among sites, among-collection dates within sites, and within-collection dates at sites (Table 6). Nearly 88% of the total variance fell within collections, quantifying our findings from dendrograms of all 1357 plants (dendrograms not shown) that the diversity existing within collections was much greater than that associated with sites or collection dates within sites. The variance among sites was slightly larger than the variance among collections within sites. When data from the Bowers field were analyzed separately, the six residue management treatments that had been imposed for 3 consecutive years prior to our sampling had no effect on the genetic structure of the *P. annua* population (Fig. 1 and Table 6). The within-collection dates and among-collection dates within-residue treatment components were highly significant. Indeed, the among collections within-residue treatments component was much larger at Bowers, where it averaged 9% of the variance (Table 6) than at the ten other sites, where it averaged only 2% of the variance.

Data were partitioned into the most-commonly occurring haplotypes (those found in more than one individual in the whole population) and the least common types (Ta-

Table 6 Hierarchical analysis of molecular variance for various data groupings

Data grouping	Variance component	Variance	% Total	Prob.[f]	Φ-statistics
All individual[a]	Among sites	σ^2_a=0.161	7.5	<0.001	Φ_{CT}=0.075
	Among collections/sites	σ^{2b}=0.104	1.84	<0.001	Φ_{SC}=0.052
	Within collections	σ^2_c=1.887	87.64	<0.001	Φ_{ST}=0.123
Bowers all treatments[b]	Among treatments	σ^2_a=–0.079	–3.37	1.00	Φ_{CT}=-0.034
	Among collections/treatments	σ^2_b=0.219	9.29	<=0.001	Φ_{SC}=0.090
	Within collections	σ^2_c=2.214	94.08	<=0.001	Φ_{ST}=0.059
Other ten sites	Among sites	σ^2_a=0.208	10.52	<=0.001	Φ_{CT}=0.105
	Among collections/sites	σ^2_b=0.045	2.28	<=0.001	Φ_{SC}=0.025
	Within collections	σ^2_c=1.726	87.20	<=0.001	Φ_{ST}=0.128
Most common 184 haplotypes[d]	Among sites	σ^2_a=0.187	10.17	<=0.001	Φ_{CT}=0.102
	Among collections/sites	σ^2_b=0.084	4.58	<=0.001	Φ_{SC}=0.051
	Within collections	σ^2_c=1.567	85.25	<=0.001	Φ_{ST}=0.147
Least common 312 haplotypes[a]	Among sites	σ^2_a=0.103	3.51	<=0.003	Φ_{CT}=0.035
	Among collections/sites	σ^2_b=0.134	4.58	<=0.001	Φ_{SC}=0.047
	Within collections	σ^2_c=2.698	91.91	<=0.001	Φ_{ST}=0.081

[a] 496 *P. annua* haplotypes, all sites
[b] 258 *P. annua* haplotypes at Bowers
[c] 324 *P. annua* haplotypes at all ten other sites
[d] 184 most common haplotypes (those occurring more than once in the whole population of 1357 individuals, with 1045 individuals possessing these 184 most common haplotypes
[e] 312 least common haplotypes (those that occurred only once in the whole population of 1357 individuals)
[f] Probability of more extreme variance components and Φ-statistic than the observed value by chance alone, based on 1000 random permutations. Φ_{CT}, Φ_{SC} and Φ_{ST} are F-statistic analogues for the correlations of molecular diversity within sites relative to the whole species, within collections relative to the site, and within collections relative to the whole species, respectively

ble 6). As might be expected, the size of the within-collections variance decreased for the most common types and increased for the least common types compared to the pooled analysis (Table 6). The among-collection dates within-sites component occupied 4.6% of the total variance for both groups. However, the variance among sites was much smaller in the least common group (3.5% of total) than in the most common group (10.2% of total). From a biological point of view, the most common haplotypes would generally be the ones doing best as weeds in grass seed production fields. These most common types show highly significant effects among sites because each field has had a unique history of selection pressure and weed seed importation, creating its own unique mix of successful, dominant types. The significant within-collections effect occurs as a result of the background diversity within this species, the selection pressure at each site, and some degree of genetic control over germination patterns. The small size of the among-collections within-sites variance may imply that among the traits of this highly successful weed is a tendency to maintain only relatively modest genetic barriers between fall-, winter-, and spring-germinating types. The least common types may be those not faring as well under current grass seed production practices, and may represent the background population diversity in Willamette Valley *P. annua*, the gene pool from which the more successful types have arisen. It is likely that the most abundant genotypes in commercial production fields are those that tolerate herbicide and residue management treatments imposed by growers. However, because we collected our seedlings at the two-leaf growth stage, we may have "rescued" some seedlings that would have otherwise succumbed to diuron present in the field soil.

Discussion

Dendrograms and principal coordinate analysis (PCA) plots of the entire data set, or large subsets of it, were extremely complex, and failed to concisely summarize the data. Individuals from any particular site were scattered over a large portion of the trees or PCA plots. The UPGMA algorithm produced large numbers of tied trees, and tree nodes were often highly multifurcating. However, when individuals were structured as populations based on site and date of collection, and analyzed either by POPGENE or AMOVA, the UPGMA algorithm resulted in trees whose populations clustered based on their respective herbicide history and season of germination.

The mean effective number of alleles for all individuals and all loci was n_e=1.4. According to Kimura and Crow (1964) and Hartl and Clark (1989), the effective number of alleles estimates the reciprocal of homozygosity. Based on this estimate, homozygosity of the *P. annua* populations was about 71%. This homozygosity is close to what the literature implies for 22% outcrossing in *P. annua*. The fact that progenies derived from seeds of single plants segregated for diuron resistance in greenhouse studies (Mengistu 1999, Mengistu et al. 2000), along with a within-population diversity of 91–96%, support an estimate of up to 29% outcrossing in our populations. A survey by Schoen and Brown (1991) of genetic diversity in published isozyme data for eight self-fertilizing species found mean h=0.125, minimum h=0.008, maximum h=0.294, and range of h=0.286, while for nine outcrossing species mean h=0.257, minimum h=0.174, maximum h=0.328, and range of h=0.154. Nei's gene diversity for our *P. annua* gave mean h=0.241, minimum h=0.119, maximum h=0.293 and range of h=0.174. The

gene diversity statistics of *P. annua* better resemble those of outcrossing species than of self-fertilizing species. The genetic difference among 47 populations of *P. annua* in western Oregon in our study (G_{ST}=0.146) is far less than seen in self-pollinating species, where the reported G_{ST}=0.51 (Hamrick and Godt 1989). Our value, however, is in close accord with $G_{ST=}$0.1 to 0.22 for mixed mating and 0.10 to 0.20 for outcrossing species (Hamrick and Godt 1989). This may have been caused by high outcrossing rates in our populations that maintained diversity within the species.

Comparing results from AMOVA (Table 6) with that of the Nei gene diversity analysis and differentiation parameters (Table 5), the diversity among the 16 sites was G_{ST}=0.087, while the within-site diversity was G_{ST}= 0.065, indicating that population differentiation among sites was higher than population differentiation within site for 16 sites and 47 populations. The greatest diversity among collection dates within sites was at Bowers and at Hyslop, with G_{ST} values of 0.125 and 0.113, respectively. The lowest diversity was at Pugh perennial ryegrass, Glaser meadowfoam, and Coon full straw chop, with G_{ST} values of 0.016, 0.021 and 0.030, respectively (Table 4). Other diversity parameters like h, H_T, and H_S all showed similar patterns across sites, where the maximum values of diversity were found at Bowers and Hyslop and the minimum values were at Glaser meadowfoam and Coon full straw chop. The diversity statistics of the remaining sites fell between these two extremes, similar to what was seen in the AMOVA analysis (Table 6).

We obtain roughly similar estimates of the collection-date effect at Bowers using either AMOVA (Table 6), where Φ_{SC}=0.09, or POPGENE (Table 5), where G_{CS}=0.0858 when grouped by the six residue management treatments and G_{ST}=0.0667 when grouped by collection dates. However, there was some discrepancy in the percentage partitioning, possibly because of the negative percentage for Φ_{CT} calculated by AMOVA. Furthermore, the percentage in POPGENE is for only two hierarchies, which were either among and within residue treatments or among and within collection dates, as opposed to AMOVA, which handled three levels of hierarchy in a single analysis.

Distribution of genetic diversity showed patterns in accord with herbicide histories and selection pressures at specific sites. For example, the 8 out of 16 sites that showed G_{ST} <0.05 are those with low gene diversity, and are dominated by herbicide-resistant genotypes, with only 46 to 65% of the individuals susceptible to diuron (Mengistu 1999, Mengistu et al. 2000). The 8 out of 16 sites with G_{ST} values between 0.05 and 0.15 had experienced lower levels of selection pressure, and 83 to 94% of the individuals remained susceptible to diuron (Mengistu 1999). We have no reason to expect significant variation in mutation rates or drift across populations. However, we knew that the herbicide selection pressure varied across sites even before we sampled them. The larger diversity statistics and differentiation values at Bowers and Hyslop and the smaller values at the other sites are in agreement with the concept that population diversity of *P. annua* of western Oregon grass seed crops has been shaped by selection pressure from herbicides.

The largest calculated rate of gene flow among collection dates was for Pugh perennial ryegrass (N_m=15.2), while the smallest was for Bowers bale (N_m=1.53) (Table 4). Highest values of N_m among collection dates occurred at sites of high selection pressure and lowest values occurred at Hyslop and Bowers, fields on which fewer grass seed crops were grown over the past four decades than at the other sites. Estimated gene flow can be used as an indicator of selection, with the trend matching the diuron-use histories at these sites. The ratio of individual N_m values at each site to the maximum N_m at Pugh perennial ryegrass (15.2) produces indices of relative selection intensity. These indices ranged from 0.1 at Bowers bale and 0.13 at Hyslop bale/flail/rake to 0.78 at Glaser meadowfoam and 1.0 at Pugh perennial ryegrass.

Our findings also agree with those of Darmency and Gasquez (1983) where herbicide-susceptible populations were more diverse than resistant populations. Warwick and Black (1993) found that the total diversity in triazine-resistant *Brassica rapa* populations (H_T=0.174) was smaller than in susceptible populations (H_T=0.191), the same trend that we saw. However, gene diversity among their resistant populations (G_{ST}=0.121), and among their susceptible populations (G_{ST}=0.037), is in disagreement with our results, where we found greater diversity among susceptible populations (G_{ST}=0.125) than among resistant populations (G_{ST}=0.016).

The founding populations of western Oregon *P. annua* must have been rich in diversity. Although herbicide selection pressure has narrowed this diversity somewhat at sites of intensive grass seed production, this reduction in diversity most likely reflects dilution of the gene pool by the high population density of relatively few resistant types rather than any true loss of diversity from the soil seed bank. The implications of the large genetic diversity of *P. annua* on sustainable weed-control practices in grass seed production are still under consideration. This weed has apparently evolved resistance to herbicides on multiple occasions in many different fields. Common-sense sanitation practices, such as removing weed seed from equipment between fields and planting weed-free seed, will not prevent this species from evolving resistance to new herbicides, but should slow the development of biotypes stacking multiple resistance mechanisms into single “super-weeds.” Any successful resistance management strategy for *P. annua* must also limit gene flow between the now abundant herbicide-resistant biotypes and the diverse but generally susceptible types present in the soil seed bank.

Acknowledgements The authors thank the Oregon Seed Council for partial funding of this project. Contribution of the Agricultural Research Service, USDA, in cooperation with the Agricultural Experimental Station, Oregon State University Techical Paper No. 11540 of the latter. Experiments were conducted in compliance with all laws of the state of Oregon and the United States.

References

Bassam BJ, Gustavo CA, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Chong D K X, Yang RC, Yeh FC (1994) Nucleotide divergence between populations of trembling aspen (*Populus tremuloides*) estimated with RAPDs. Curr Genet 26:374–376

Darmency H, Gasquez J (1981) Inheritance of triazine resistance in *Poa annua*: consequences for population dynamics. New Phytol 89:487–493

Darmency H, Gasquez J (1983) Esterase polymorphism and growth form differentiation in *Poa annua* L. New Phytol 95: 289–297

Darmency H, Gasquez J, Matejicek A (1992) Association of esterase isozymes with morphology in F_2 progenies of two growth variants in *Poa annua* L. New Phytol 121:657–669

Ellis WM, Lee BTO, Calder DM (1970) A biometrical analysis of populations of *Poa annua* L. Evolution 25:29–37

Excoffier L P, Smouse E, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Gibeault VA, Goetze NR (1973) Annual meadow-grass. J Sports Turf Res Inst 48:9–19

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL Weir BS (eds) Plant population genetics, breeding and genetic resources. Sinauer Associates, Sunderland, Massachusetts, pp 43–63

Hartl DL, Clark AG (1989) Principles of population genetics 2nd edn. Sinauer Associates, Sunderland, Massachusetts

Heap, IM (1995) Multiple herbicide resistance in annual bluegrass (*Poa annua*). WSSA Abstracts 42:56

Holm L, Holm JDE, Pancho J, Herberger J (1997) World weeds. Natural histories and distribution. John Wiley and Sons, Inc., pp 585–595

Huff DR, Peakall R, Smouse PE (1993) RAPD variation within and among natural populations of outcrossing bufallograss [*Buchloë dactyloides* (Nutt.) Englem.] Theor Appl Genet 86:927–934

Johnson PG, White DB (1997) Flowering responses of selected annual bluegrass genotypes under different photoperiod and cold treatments. Crop Sci 37:1543–1547

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. Genetics 49:725–735

Mengistu LW (1999) Genetic diversity and herbicide resistance in annual bluegrass (*Poa annua* L.), PhD thesis, Oregon State University, Corvallis-Oregon, USA. Dissertation abstract no. AAT 9842465

Mengistu LW, Mueller-Warrant GW, Liston A, Barker RE (2000) psbA Mutation (valine$_{219}$ to isoleucine) in *Poa annua* resistant to metribuzin and diuron. Pest Management Sci 56:209–217

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA. 70:3321–3323

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590

Rohlf FJ (1993) NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.80. New York

Schoen DJ, Brown AHD (1991) Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants. Proc Natl Acad Sci USA 88:4494–449

Slatkin M, Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349–1368

Steiner JJ, Poklemba CJ, Fjellstrom RG, Elliott LF (1995) A rapid one-tube genomic DNA extraction process for PCR and RAPD analysis. Nucleic Acids Res 23:2569–2570

Sweeney P.M, Danneberger TK (1995) RAPD characterization of *Poa annua* L. populations in golf course greens and fairways. Crop Sci 35:1676–1680

Tutin T (1957) A contribution to the experimental taxonomy of *Poa annua*. Watsonia 4:1–10

Warwick S (1979) The biology of Canadian weeds. 37. *Poa annua* L. Canadian Plant Sci 59:1053–1066

Warwick S, Briggs D (1978) The genecology of lawn weeds. I. Population differentiation in *Poa annua* L. in a mosaic environment of Bowling Green lawns and flower beds. New Phytol 81:711–723

Warwick S, Black LD (1993) Electrophoretic variation in triazine-resistant and susceptible populations of the allogamous weed *Brassica rapa*. Weed Res 35:105–114

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535

Wright S (1969) Evolution and genetics of populations. The theory of gene frequencies, Vol. 2. University of Chicago Press, Chicago. Vol. 2.

Wu L, Till-bottraud I, Torres A (1987) Genetic differentiation in temperature-enforced seed dormancy among golf-course populations of *Poa annua* L. New Phytol 107:623–631

Yeh FC, Young RC, Timothy B, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canda