Theor Appl Genet (1993) 86:927–934

RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]

D. R. Huff[1], R. Peakall[1,2], P. E. Smouse[3]

[1] Department of Crop Science, Cook College, Rutgers University, New Brunswick, New Jersey 08903-0231, USA
[2] School of Biological Sciences, Macquarie University, NSW 2109 Australia
[3] Center for Theoretical and Applied Genetics (CTAG), Cook College, Rutgers University, New Brunswick, NJ 08903-0231, USA

Received: 16 September 1992 / Accepted: 28 January 1993

Abstract. RAPD markers provide a powerful tool for the investigation of genetic variation in natural and domesticated populations. Recent studies of strain/cultivar identification have shown extensive RAPD divergence among, but little variation within, inbred species or cultivars. In contrast, little is known about the pattern and extent of RAPD variation in heterogeneous, outcrossing species. We describe the population genetic variation of RAPD markers in natural, diploid sources of dioecious buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. Buffalograss is native to the semi-arid regions of the Great Plains of North America, where it is important for rangeland forage, soil conservation, and as turfgrass. Most sources of buffalograss germplasm are polyploid; diploid populations are previously known only from semi-arid Central Mexico. This is the first report of diploids from humid Gulf Coastal Texas. These two diploid sources represent divergent adaptive ecotypes. Seven 10-mer primers produced 98 polymorphic banding sites. Based on the presence/absence of bands, a genetic distance matrix was calculated. The new Analysis of Molecular Variance (AMOVA) technique was used to apportion the variation among individuals within populations, among populations within adaptive regions, and among regions. There was considerable variation within each of the four populations, and every individual was genetically distinct. Even so, genetic divergence was found among local populations. Within-population variation was larger and among-population variation smaller in Mexico than in Texas. The largest observed genetic differences were those between the two regional ecotypes. These patterns of genetic variation were very different from those reported for inbred species and provide important baseline data for cultivar identification and continuing studies of the evolution of polyploid races in this species.

Key words: Population genetic variation – Buffalograss – AMOVA

Communicated by A. L. Kahler
Correspondence to: D. R. Huff

Introduction

The development of random amplified polymorphic DNA (RAPD) markers by Williams et al. (1990) and Welsh and McClelland (1990) has provided a powerful tool for the investigation of genetic variation. The RAPD procedure works with anonymous genomic markers, requires only small amounts of DNA, and is simpler, less costly, and less labor intensive than other DNA marker methodologies (Caetano-Anolles et al. 1991a, b; Hadrys et al. 1992). The utility of RAPDs for genetic characterization of plant populations has been demonstrated by several workers, and the number of laboratories using RAPD methodology is growing rapidly. For example, RAPDs have been used in the contruction of genetic maps in soybean (Williams et al. 1990), tomato (Klein-Lankhorst et al. 1991), and conifers (Carlson et al. 1991), to identify hybrids in maize (Welsh et al. 1991) and irises (Arnold et al. 1991), and as markers for disease resistance genes in lettuce (Michelmore et al. 1991).

RAPDs can readily identify germplasm sources of cocoa (Wilde et al. 1992), commercial cultivars of broccoli and cauliflower (Hu and Quiros 1991); while Kazan et al. (1992) have used them to construct a phylogeny of

legume cultivars in the genus *Stylosanthes*. These latter studies have shown extensive RAPD divergence among, but little variation within, inbred species or cultivars. In contrast, little is known about the pattern and extent of RAPD variation in heterogeneous, outcrossing species. An understanding of such patterns is important if we are to utilize RAPDs for the identification of germplasm sources and open-pollinated cultivars.

We describe the population genetic variation of RAPD markers in natural sources of dioecious buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. Buffalograss is native to the semi-arid regions of the Great Plains of North America, where it is important for rangeland forage, soil conservation, and as turfgrass (Huff and Wu 1992). Most sources of buffalograss germplasm are polyploid ($2n = 40$ or 60). Diploid populations provide an important genetic resource for crop improvement (Huff 1991), but are known only from two distinct regions, one located in semi-arid Central Mexico (Reeder 1971), annual precipitation ≤ 38 cm, and the other along the semi-tropical Gulf Coast of southeastern Texas, annual precipitation ≥ 127 cm. This is the first report of the Texas diploids. From our observations, these two diploid sources represent divergent adaptive types, from which the more widespread polyploids may have evolved.

This paper provides important baseline data on the RAPD genetic variation useful for germplasm and cultivar identification and for continuing studies of the evolution of the polyploid races. At the same time, we are the first to apply the recently developed analysis of molecular variance, AMOVA (Excoffier et al. 1992), to RAPD phenotypes, apportioning genetic variation among individuals/within populations, among populations/within adaptive regions, and among regions (e.g., Texas versus Mexico). While AMOVA was developed specifically for RFLP data, we show that RAPD data can easily be accommodated. Our specific objectives are to determine the pattern and extent of RAPD marker variation within and among natural populations of an obligate outcrossing species, and to determine the usefulness of RAPD markers for germplasm monitoring and for the identification of open-pollinated cultivars.

Materials and methods

Population sampling

Either stolon or seed samples were collected from four natural populations of buffalograss (Fig. 1). Two of the four populations, located approximately 70 km apart, were sampled as seed from San Luis Potosi, Mexico. Each Mexican population was derived from half-sib seed collected from three individual female plants, separated by distances of 40 to 50 m, within an approximately 5-ha area at each location. The other two populations, located approximately 7 km apart, were sampled as vegetative stolons from Matagorda country, Texas. Each Texas population was derived from individual vegetative stolons, collected at intervals of 50 to 150 m, within an approximately 10-ha area at each location. In addition to the adaptive genetic differences that are probably due to the climatic differences between humid Texas and semi-arid Mexico, Mexico was represented by sets of half-siblings, whereas Texas was represented by a set of (probably) unrelated individuals. Plant materials from all four populations were established in the experimental field at Cook College, Rutgers University.

Template DNA isolation

Template DNA was prepared by grinding approximately 300 mg of fresh leaf tissue in liquid nitrogen, placing 0.6 ml vol of ground tissue into a 1.7-ml microfuge tube, adding 1/3 vol buffer (100 mM Tris; 50 mM EDTA, pH 7.5; 100 mM NaCl; 1% SDS; 10 mM mercaptoethanol; 0.1% PVP) and 1/3 vol 5 M potassium acetate. Tubes were mixed with a sterile pipet tip, incubated in a 65 °C water bath for 15 min, then on ice for 10 min, and then spun at 13 kg for 20 min. Supernatant was transferred into clean tubes, to which was added 1/5 vol of 10 M ammonium acetate and 1 vol −20 °C isopropanol. Tubes were gently mixed and stored at −20 °C for 20 min. The DNA was pelleted, washed in 70% ethanol, and dissolved in 100 µl of double-distilled H_2O. The DNA content of each sample was measured using a TKO-100 fluorometer and diluted to 10 ng μl^{-1}.

DNA amplification

DNA amplification was performed in a Perkin-Elmer Cetus DNA-Thermocycler. The optimal program for buffalograss

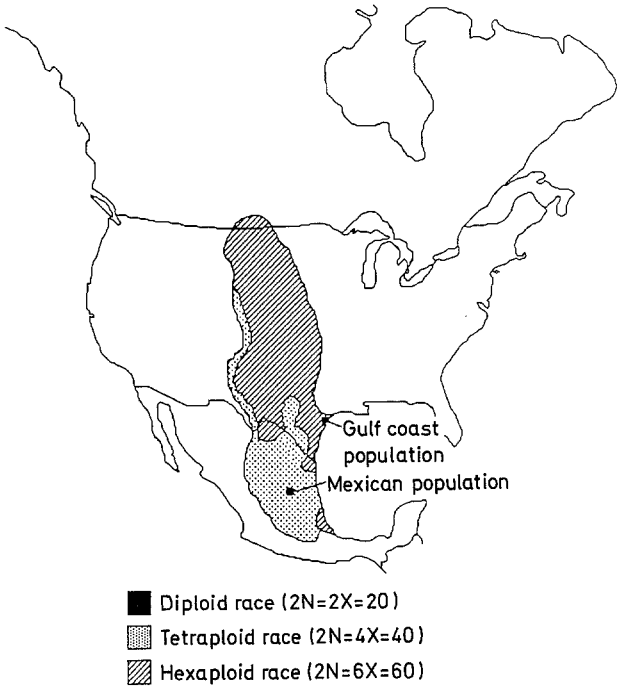

Fig. 1. Geographic range of buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.], showing locations of the four diploid population samples collected from Texas (Texas-A and Texas-B) and Mexico (Mexico-A and Mexico-B)

commenced with 7 min at 94 °C, followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C, terminated with 5 min at 72 °C. Ramp times for heating and cooling were 3 degrees/s and 1 degree/s, respectively. Reactions were carried out in a 12 µl vol containing 1 X buffer (Promega), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µm primer, 0.024 U μl^{-1} *Taq* polymerase (Promega), and 1 ng μl^{-1} template DNA. DNA amplification fragments were separated in 7.5% acrylamide/bis gel (Bio-Rad) in 0.375 M Tris, pH 8.8 buffer, using a Mini-Protean II. Fragments were then visualized with silver stain (Bio-Rad kit), following the recommended protocol, with the exception that the fixation step was performed in 10% acetic acid for 30 min.

Twenty Operon set-A (OPA) 10-mer primers (Operon Technologies, Inc., Alameda, Calif.) were evaluated for suitability in a pilot survey. Seven primers (OPA-5, OPA-7, OPA-9, OPA-10, OPA-13, OPA-19, OPA-20) gave reproducible and informative marker patterns and were selected for the final study. Band fragments included in the final analysis ranged between 400 bp to 2.5 kb in length and were scored for presence or absence (Fig. 2). RAPD markers generated by the seven OPA primers that were consistently reproduced in at least two replicate PCR reactions and that were reproducible across DNA extractions were included in the analysis. To aid interpretation of homology, each 15-lane gel consisted of six individuals from two populations and three equally spaced lanes of PGem *Hin*dIII marker DNA. Replicate gels consisted of alternating side-by-side comparisons of the four populations. The presence or absence of homologous bands was determined for all individuals and a matrix of RAPD phenotypes was assembled. Each individual was represented by a vector of 1 s and 0 s, 1 for the presence of any particular band and 0 for its absence.

Statistical analysis

After excluding markers that were monomorphic for the entire data set, we used the vector of marker presence/absence states for each individual to compute two measures of genetic distance for all pairs of individuals. The first was a slightly modified distance metric from Nei and Li (1979)

$$\mathbf{D} = \{\delta^2_{xy}\} = 100\left[1 - \frac{2n_{xy}}{n_x + n_y}\right] = 100(1 - F_{xy}), \quad (1)$$

where n_x and n_y are the numbers of markers observed in individuals x and y, respectively, and $2n_{xy}$ is the number of markers shared by the two individuals; multiplication by 100 merely puts the number on the same scale as the second measure. The seccond measure was the Euclidean metric of Excoffier et al. (1992), defined here (in analogous terms) as

$$\mathbf{E} = \{\varepsilon^2_{xy}\} = n\left[1 - \frac{2n_{xy}}{2n}\right], \quad (2)$$

where n is the total number of polymorphic sites. This latter measure amounts to a tally of band differences between individuals.

We used the new Analysis of Molecular Variance (AMOVA) procedure (Excoffier et al. 1992) to estimate variance components for RAPD phenotypes, partitioning the variation among individuals/within populations, among populations/within adaptive regions, and among regions (Texas versus Mexico). The central idea of AMOVA is to convert the 48 × 48 inter-individual distance matrix **E** into an equivalent analysis of variance, the essential pattern for which is shown in Table 1. A classic variance-components extraction yields the variance components of interest. Significance level for variance component estimates were computed by non-parametric permutational procedures. Both the non-Euclidean $\mathbf{D} = \{\delta^2_{xy}\}$ and Euclidean $\mathbf{E} = \{\varepsilon^2_{xy}\}$ distance matrices were subjected to analysis. AMOVA is not strictly rigorous with a non-Euclidean metric, but δ^2_{xy} and ε^2_{xy} are empirically similar for these data, and as pointed out by Excoffier et al. (1992), the two measures differ only in the choice of denominator, $(n_x + n_y)$ versus $2n$; the distance metrics are nearly interchangeable, and we will report only the Euclidean measure here. We analysed both the total data sets and the separate data sets within Texas and Mexico, and also reduced data sets for each of the individual RAPD primers. All analyses were undertaken with the WINAMOVA program provided by Laurent Excoffier (Department of Anthropology and Ecology, University of Geneva, 12, rue G. Reviliode, 1227 Carouge, Switzerland).

Relationships among the 48 individuals were represented in dendrogramic form, using the distance matrices **D** and **E** with the average linkage procedure of SYSTAT. Again, the results emerging from the two distance metrics were virtually interchangeable; we present only those from the Euclidean measure. The sample sizes are small, and we have not elicited all of the variation, but the results clarify the taxonomic/identification situation considerably.

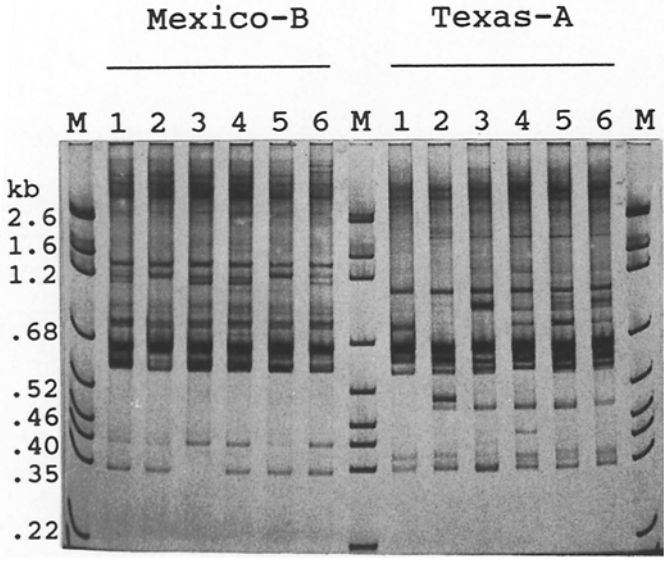

Fig. 2. Polyacrylamide gel of silver-stained RAPD markers generated with primer OPA-13 for buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.], six individuals from the Mexico-B population and six from the Texas-A population

Results

The RAPD profile

After excluding bands that were monomorphic for the whole data set, the seven primers yielded a total of 98 polymorphic markers (Table 2). The number of markers ranged from six (OPA-7) to 29 (OPA-10) and was positively correlated ($r = 0.974$, $P < 0.01$) with the number of unique phenotypes identified by each primer. Of the 98 RAPD markers scored, only seven (7%) of the markers showed fixed differences among regions, while 39 (40%) were variable in both regions, and 52 (53%) were variable in one region but absent from the other. However, most of the divergence among regions was attributable to markers of variable frequency, rather than to the markers exhibiting fixed differences. Of the 62 markers present within the Texas populations, six (9.7%) were monomorphic, none (0%) were fixed in either population, 37 (59.7%) were variable in both populations, and 19 (30.6%) were variable in one population but absent from the other. Of the 68 markers present within the Mexico populations, 13 (19.1%) were monomorphic, one (1.5%) was fixed in one population but absent from the other, 38 (55.9%) were variable in both populations, and 16 (23.5%) were variable in one population but absent from the other. Because fixed differences among regions and among populations/within regions were few, the AMOVA results were based, in large part, on the frequency differences of variable RAPD markers.

Table 1. Molecular analysis of variance format for the extraction of components of RAPD variation among regions, among populations within region, and among individuals within populations

Source of variation	*df*	Mean squared deviation	E(MSD)
Regional differences	1	MSD(a)	$\sigma_c^2 + 12\sigma_b^2 + 24\sigma_a^2$
Populations/ regions	2	MSD(b)	$\sigma_c^2 + 12\sigma_b^2$
Individuals/ populations	44	MSD(c)	σ_c^2

The distance matrix

All of the analyses that follow, as well as others that one might pursue, can be derived from this same RAPD phenotypic distance matrix, **E** (Table 3). Even a casual perusal of the distance matrix will elicit four salient points of the data set. (1) There is considerable variation within each of the four populations, and every individual is genetically unique. This is very different from the usual result with inbred strains of other species, but is what we might anticipate from an obligate outbred organism. (2) In spite of substantial internal variation, there is measureable divergence among local populations. Individuals in different populations of the same region are more divergent, on the average, than are individuals within the same population. (3) Within-population variation is larger and among-population variation smaller in Mexico than in Texas. This could be due to the difference in sampling procedure (half-sib families versus unrelated individuals) or to real differences in genetic population structure between the two regions. (4) The differences between the two regional ecotypes are so striking as to be categorical, far exceeding the seven fixed differences we alluded to above (Table 2). Given these results, we have reason to anticipate a useful degree of taxonomic resolution.

Table 2. Attributes of oligonucleotide primers used for generating RAPD markers from 48 individuals of *Buchloë dactyloides* (Nutt.) Engelm. sampled from two populations from each of two regions (Texas and Mexico)

Primer	Nucleotide sequence 5′ to 3′	Number of polymorphic markers	Number of unique phenotypes	Fixed differences		
				Among regions	Within Texas	Within Mexico
OPA-5	AGGGGTCTTG	11	16	1	0	0
OPA-7	GAAACGGGTG	6	9	0	0	0
OPA-9	GGGTAACGCC	8	15	0	0	0
OPA-10	GTGATCGCAG	29	46	2	0	0
OPA-13	CAGCACCCAC	24	36	3	0	1
OPA-19	CAAACGTCGG	12	12	0	0	0
OPA-20	GTTGCGATCC	8	15	1	0	0
Total		98	48	7	0	1

Table 3. Euclidean distances between all pairs of 48 *Buchloë dactyloides* individuals, using 98 RAPD markers. The 48 individuals originate from two regions, Texas and Mexico, with each region represented by two populations (Texas-A and Texas-B, Mexico-A and Mexico-B) of 12 individuals each. (a) Genetic distances within Texas above the diagonal, and those within Mexico below the diagonal; (b) Genetic distances between Texas and Mexico

(a) Texas-A vs Texas-A

–	13	15	15	20	15	13	7	16	13	12	10
12	–	18	16	17	20	10	12	17	16	13	15
17	15	–	12	15	16	14	14	15	14	13	15
20	20	25	–	15	16	16	18	17	18	15	15
16	14	21	20	–	13	15	15	10	11	14	12
22	14	11	22	18	–	16	14	13	14	13	11
20	12	19	16	14	14	–	10	13	10	9	13
18	14	21	26	14	22	18	–	11	10	9	7
14	16	21	22	16	22	16	10	–	7	10	6
17	15	18	23	13	17	17	11	15	–	11	9
13	17	24	21	21	23	17	13	9	14	–	8
18	16	21	18	14	20	12	16	12	17	15	–

Texas-A vs Texas-B

22	19	21	17	17	19	19	20	14	17	19	17
23	20	22	18	22	20	20	21	15	18	20	18
23	20	20	18	18	20	20	23	17	18	20	18
23	16	22	14	16	16	20	21	15	14	18	16
20	19	10	13	19	15	23	18	18	17	17	15
21	22	20	18	22	20	22	21	17	20	18	18
19	12	16	16	18	16	14	19	13	16	16	16
21	18	18	14	18	18	18	19	13	16	18	14
22	19	19	15	17	15	19	19	16	13	15	13
21	16	16	16	18	14	18	13	17	18	16	16
22	17	19	13	19	19	21	18	14	13	17	13
20	21	17	13	19	19	21	18	14	13	17	13

Mexico-A vs Mexico-A

Mexico-A vs Mexico-B

19	15	22	23	17	19	15	15	13	12	10	15
20	20	19	24	20	20	18	22	20	17	17	20
18	18	21	20	20	22	16	20	18	15	13	18
20	16	19	22	20	18	16	18	18	13	15	20
18	20	17	24	18	18	16	18	16	13	13	16
14	14	21	22	12	20	16	14	16	11	13	14
15	13	16	19	13	17	13	13	11	12	14	13
23	21	26	25	25	27	21	21	21	20	22	25
17	17	18	21	17	21	13	13	13	14	14	13
17	15	20	19	15	21	13	15	13	12	14	13
16	14	17	20	14	18	12	12	12	13	13	10
18	18	17	20	18	20	14	18	16	13	15	16

Texas-B vs Texas-B

–	17	11	19	19	15	21	20	14	21	19	17
9	–	14	14	12	8	10	13	9	14	14	14
9	4	–	12	16	14	18	11	9	16	14	12
7	6	4	–	10	10	16	11	9	10	6	2
9	6	8	8	–	8	18	13	11	16	12	10
9	10	8	8	8	–	16	9	9	16	10	10
12	13	13	11	9	11	–	17	13	10	12	14
14	13	13	11	15	15	16	–	10	11	11	11
10	9	9	11	7	11	8	16	–	11	9	7
10	13	9	13	13	13	8	18	8	–	10	10
9	12	10	12	10	10	7	17	5	5	–	4
11	14	10	12	12	14	9	19	11	5	6	–

Mexico-B vs Mexico-B

(b) Texas-A vs Mexico-A

43	48	46	48	45	48	38	40	39	40	41	41
41	44	44	44	47	48	36	40	41	42	39	41
40	45	43	45	44	47	37	37	40	41	40	38
39	44	42	44	43	44	36	38	41	40	37	41
41	48	44	46	43	46	38	40	39	42	39	39
43	46	46	46	47	48	40	42	43	44	43	41
39	46	44	46	47	46	36	38	43	42	39	41
37	44	42	44	45	46	34	38	41	42	37	41
39	44	42	44	45	46	34	38	41	42	37	41
40	45	43	45	44	47	35	37	40	41	38	40
42	49	47	49	48	47	39	41	44	45	42	46
41	46	44	46	45	48	38	38	41	42	39	41

Texas-B vs Mexico-A

39	40	40	40	42	40	38	39	39	38	36	38
37	38	42	42	40	40	34	41	39	36	38	40
40	43	41	39	43	43	39	42	40	37	41	39
43	42	44	40	40	40	40	41	39	40	36	40
39	40	42	38	38	38	38	41	39	38	36	36
39	44	40	40	40	40	40	41	41	40	40	40
39	40	40	40	40	40	34	41	39	38	38	40
37	36	42	40	38	38	36	41	37	38	38	38
37	36	40	38	36	38	34	39	35	36	34	36
38	37	41	37	37	37	35	40	36	37	35	35
38	39	43	43	41	39	37	42	40	41	37	41
39	38	44	38	38	38	36	41	37	38	36	36

Texas-A vs Mexico-B

42	49	47	49	48	47	39	41	44	45	42	44
43	50	48	50	47	48	42	40	45	46	45	43
43	50	48	50	49	50	40	40	47	46	43	45
43	50	48	50	51	50	40	42	49	48	43	47
41	48	46	48	45	46	38	38	43	42	41	41
39	46	44	46	45	46	36	36	41	42	39	41
42	47	45	47	48	49	37	41	44	45	40	44
40	49	47	47	50	49	41	43	50	47	44	46
40	45	43	45	44	47	35	37	42	41	38	40
44	47	47	49	46	49	37	39	42	43	40	42
45	50	48	50	49	52	40	42	45	46	43	45
43	50	48	50	49	50	40	40	45	46	43	45

Texas-B vs Mexico-B

38	41	43	41	39	39	37	42	40	41	37	39
41	46	44	40	42	42	42	45	43	44	42	40
43	44	46	42	44	44	40	47	43	44	42	42
43	44	46	44	44	44	40	47	43	44	42	44
39	42	42	38	40	40	38	43	41	42	38	38
39	38	42	38	38	38	34	41	37	38	36	36
38	39	43	41	39	39	37	42	38	39	37	39
44	45	49	47	43	45	43	45	44	47	45	47
38	39	41	37	39	39	39	42	38	41	39	37
38	39	43	41	41	39	39	42	38	41	39	39
41	42	46	42	42	42	40	45	41	42	40	40
43	44	46	42	44	44	40	45	41	42	40	40

Table 4. Analysis of Molecular Variance (AMOVA) for 48 individuals of *Buchloë dactyloides*, using 98 RAPD markers. The total data set contains individuals from two regions, Texas and Mexico, each represented by two population samples of 12 individuals each. AMOVAs were also performed for the 24 individuals within each region, using 62 RAPDs for Texas and 68 for Mexico. Statistics include sums of squared deviations (SSDs), mean squared deviations (MSDs), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (*P*) of obtaining a more extreme component estimate by chance alone

Source of variation	*df*	SSD	MSD	Variance component	% Total	*P*-value
Texas vs Mexico	1	323.58	323.58	12.19	58.4	< 0.001
Populations/regions	2	62.08	31.04	2.03	9.7	< 0.001
Individuals/populations	44	293.17	6.66	6.66	31.9	< 0.001
Populations/Texas	1	32.25	32.25	2.40	27.1	< 0.001
Individuals/populations	22	142.00	6.46	6.46	72.9	< 0.001
Populations/Mexico	1	26.83	26.83	1.66	19.5	< 0.001
Individuals/populations	22	151.17	6.87	6.87	80.5	< 0.001

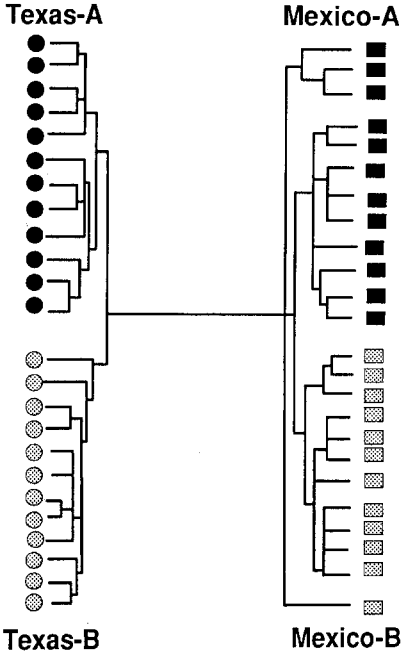

Fig. 3. Dendrogram analysis for 48 individuals of buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.] using 98 RAPD markers. The 48 individuals originated from two regions, Texas and Mexico. Each region was represented by two populations (Texas-A and Texas-B, Mexico-A and Mexico-B) and by 12 individuals for each population

The AMOVA partition

From the distance matrix, we extract an AMOVA, demonstrating highly significant ($P < 0.001$) genetic differences between Texan and Mexican regional collections, as well as between populations within either adaptive region (Table 4). There is, nevertheless, substantial variation within each of the four populations. These results were expected, given an inspection of Table 3, and are entirely in keeping with what we might expect of an obligate outcrossing organism. Of the total genetic diversity, 58.4% was attributable to regional divergence, 9.7% to population differences within regions, and 31.9% to individual differences within a population. For the within-region analyses, most of the genetic diversity was attributable to differences among individuals within a population, 72.9% for Texas and 80.5% for Mexico.

A taxonomic tree

An unrooted dendrogram, based on the average linkage algorithim, separates individuals from the two adaptive regions (Fig. 3). The two populations from Texas are clearly separated, even though we have so far encountered no fixed differences between them. The two populations from Mexico are also cleanly separable, but were not clearly bifurcating; in spite of one fixed difference between them (OPA-13 in Table 2). Moreover, statistical resolution was found to be less for populations in Mexico than for those in Texas (Table 4). Whether this is due to the different sampling frames (half-sib sets from Mexico versus unrelated individuals from Texas) or whether it reflects differential population structure in the two regions must remain an open question, pending further sampling. Inasmuch as these individuals are members of interbreeding gene pools, or perhaps even reproductively-connected gene pools (at least within a region), the dendrogram is best viewed as a simple representation of the relative scales of variation within and among populations, compared to that between regional ecotypes. In any event, each of the 48 individuals exhibits a unique diploid phenotype. We can therefore state that the four gene pools represent non-overlapping portions of the 98-dimensional genetic space and that populations within a region occupy closely neighboring portions, while the regional ecotypes occupy very distant portions of that space. Given the climatic dif-

ferences between Texas and Mexico, this result was expected.

Discussion

This is the first paper to report RAPD variation within and among natural populations of an outcrossing species. Large genetic differences were apparent between regions, and differences between populations within an adaptive region, although smaller, were also detectable. Within populations, each individual had a unique fingerprint. Our results suggest that reports of little or no RAPD variation within populations and cultivars may be a reflection of the isogenic state or highly inbred nature of the plant materials studied heretofore, rather than an inability of the RAPD technique to identify variation within populations when it exists. This paper demonstrates the power of AMOVA in separating important and interesting regional and populational differences against a background of extensive within-population polymorphism, such as that resulting from the polyacrylamide separation of silver-stained RAPD markers from outbreeding individuals. Because only one fixed difference was observed between populations within a region, resolution derives from the cumulative impact of allele frequency differences among populations. Our sample sizes, though small ($N = 12$ per population), were large enough to establish the pattern of variation unequivocally. Future efforts to characterize population to population variation within regional biotypes should probably use somewhat larger sample sizes, perhaps as many as 25 individuals within each population. For regional differences, our sample sizes were more than adequate; the cumulative impact of frequency differences for polymorphic markers far exceeds that of the markers showing fixed differences. Decisions concerning sample sizes will, of course, depend critically on the real distributions of variation in natural populations, distributions that can be expected to vary among species. The only way to determine the appropriate sample sizes is by a preliminary survey.

Such information is useful for researchers, breeders, and seed producers who are interested in more precise monitoring and control of germplasm sources and open-pollinated cultivars. Moreover, a large number of RAPD markers may not be required to accomplish the task of 'strain-identification'. RAPD markers generated by the seven individual primers showed differential effects on the overall results of variance paritioning. Each single primer yielded a RAPD phenotype vector of reduced length, which we converted into a corresponding distance matrix, **E**, which was then subjected to the AMOVA procedure. Each of these single-primer analyses yields highly significant regional and among-population components. The distance matrix for the total data set is simply the sum of these separate single-primer matrices. Each of the variance partitions can be converted to fractional form (Fig. 4). For simple strain identification, scale will be less important than the relative apportionment of variation. The fractional partitions from OPA-5, OPA-10, OPA-13, OPA-19, and OPA-20 were similar to those of the total data set; those from OPA-7 and OPA-9 were strikingly different.

Our results suggest that it may be important to choose judiciously among the available primers for strain/cultivar identification purposes. In the current case, for example, the separation of the two regional ecotypes is so categorical that a single primer (either OPA-10 or OPA-13) is more than sufficient. Further separation of the regional ecotypes with additional primers would seem to be less rewarding than using a set of primers that would improve resolution between the two Texan populations or the two Mexican populations. The primers providing resolution for Texas might very well be different from those providing resolution for Mexico. The fact that OPA-7, with the smallest number (six) of polymorphic markers (none of which showed fixed differences among populations), was capable of separating regional and populational differences shows the utility of RAPD analysis for any research or breeding program that requires monitoring and identification of heterogeneous, outbreeding plant populations. On the other hand, a breeder interested in linkage studies might prefer primers that were highly polymorphic within all populations, possibly sacrific-

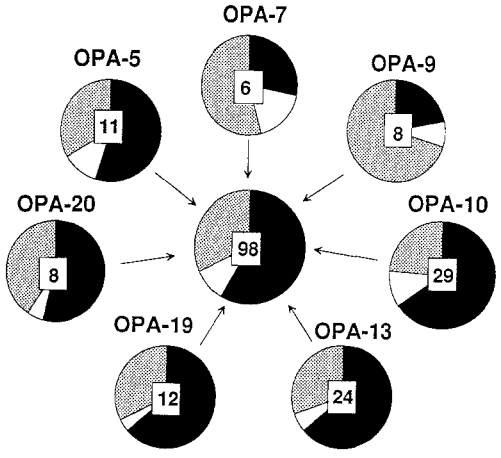

Fig. 4. Pie charts for individual RAPD primers depicting the proportions of the total variance attributable to regional differences (■), to population differences within a region (▩), and to variation among individuals within a population (□). The *numbers* in the centers of the pies are the numbers of polymorphic markers observed for the primers in question. The central figure reprsents the result of pooling all seven primers and 98 polymorphic RAPD markers

ing strain resolution in favour of crossing flexibility. We have used a small subset (seven) of the 20 primers initially tried. Given that there are thousands of primers and primer combinations possible, we should be able to design an appropriate set to address the particular problem at hand.

Acknowledgments. We thank Janice Bara for laboratory assistance, Carol Kobak for statistical assistance, and Laurent Excoffier for providing WINAMOVA. D.R.H. was funded by the Cultivated Sod Association of New Jersey, the New Jersey Turfgrass Association, the Center for Interdisciplinary Studies in Turfgrass Science, and the NJAES Project D-15264-1-93. R.P. was funded by an Australian Research Council Fellowship and wishes to thank Andrew Beattie and Steven Handel for making this collaboration possible. P.E.S. was funded by NJAES/USDA-32102 and NSF-B5R-90-06589.

References

Arnold ML, Buckner CM, Robinson JJ (1991) Pollen-mediated introgression and hybrid speciation in Louisiana irises. Proc Natl Acad Sci USA 88:1398–1402

Caetano-Anolles G, Bassam BJ, Gresshoff PM (1991a) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology 9:553–556

Caetano-Anolles G, Bassam BJ, Gresshoff PM (1991b) DNA amplification fingerprinting: a strategy for genome analysis. Plant Mol Biol Rep 9:294–307

Carlson JE, Tulsieram LK, Glaubitz JC, Luk VWK, Kauffeldt C, Rutledge R (1991) Segregation of random amplified DNA markers in F_1 progeny of conifers. Theor Appl Genet 83:194–200

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

Hadrys H, Balick M, Schierwater B (1992) Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol Ecol 1:55–63

Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Huff DR (1991) Sex ratios and inheritance of anther and stigma color in diploid buffalograss. Crop Sci 31:328–332

Huff DR, Wu L (1992) Distribution and inheritance of inconstant sex forms in natural populations of dioecious buffalograss (*Buchloë dactyloides*). Am J Bot 79:207–215

Kazan K, Manners JM, Cameron DF (1992) Genetic variation in agronomically important species of *Stylosanthes* determined using random amplified polymorphic DNA markers. Theor Appl Genet 85:882–888

Klein-Lankhorst RM, Vermunt A, Weide R, Liharska T, Zabel P (1991) Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor Appl Genet 83:108–114

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Reeder JR (1971) Notes on Mexican grasses. IX. Miscellaneous chromosome numbers. Brittonia 23:105–117

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

Welsh J, Honeycutt RJ, McClelland M, Sobral BWS (1991) Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). Theor Appl Genet 82:473–476

Wilde J, Waugh R, Powell W (1992) Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor Appl Genet 83:871–877

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