GENETIC DIVERSITY IN THE NATIVE AUSTRALIAN GRASS *AGROSTIS ADAMSONII* (POACEAE)

E.A. James[1] and A. Brown[2]

[1]Royal Botanic Gardens Melbourne, Birdwood Avenue, South Yarra, Victoria 3141, Australia

[2]State Chemistry Laboratory, Department of Natural Resources and Environment, cnr Sneyde's and South Rds, Werribee, Victoria 3030, Australia

ABSTRACT

Genetic variability in *A.adamsonii* is assessed from measurements of mature florets and from analysis of variation in DNA using the random amplified polymorphic DNA (RAPD) technique. Results are compared to those for three other species of *Agrostis* growing at the same site or in similar habitats.

KEYWORDS

Native pasture, RAPDs, conservation, bentgrass, habitat fragmentation

INTRODUCTION

In Victoria, native grasslands have been severely depleted since European settlement and now exist only as isolated remnants. Amongst the smallest remnants are specialised communities located on seasonally inundated flats in the midst of agricultural land. Their continued existence is threatened by increased drainage and grazing and by weed invasion.

In 1985, the rare endemic grass *Agrostis adamsonii* (Adamson's bent) was rediscovered in Victoria after its last recorded sighting in 1853. After considerable searching, it is now known from approximately 30 sites in the Western District of Victoria, (Fig 1) on saline, seasonally wet areas on or near the volcanic plain (Flora of Victoria, 1994). *A. adamsonii* is one of twenty-two species of *Agrostis* found in Australia and fourteen species found in Victoria, including three exotic species which have become naturalised.

DNA analysis was performed to a) examine the population genetic structure of *A. adamsonii* and b) compare the diversity of *A. adamsonii* with the other native *Agrostis* species occurring within similar environments. Morphological measurements of seed from four *Agrostis* species were collected to help in assessing diversity.

This project is part of a program to provide baseline information on the genetic conservation of *A. adamsonii*, the viability of existing populations at known sites and guidance for preferred reserves and site restoration.

MATERIALS AND METHODS

Seed collection, germination and mature floret morphology. Seeds were collected from 10 plants of *A. adamsonii* at each of 6 sites. Seeds were also collected from 10 plants from single sites of *A. aemula* var. *aemula, A. avenacea* (weeping form), *A. avenacea* (small, erect form), *A. billardierei* var. *robusta* and *A. billardierei* var. *filifolia.* Harvested seed still retained lemmas, awns and paleas but glumes had become separated in most cases.

Five seeds from five plants of each population (25 seeds per population) were measured to the nearest 1 mm for the following mature floret characters; length of lemma body, lemma setae, awn, palea, rachilla extension (including hairs), hairiness of the lemma and height of the awn attachment on the lemma back (all measurements were taken from the tip of the callus).

Twenty seeds from five plants of each population were germinated in a temperature-controlled glasshouse in 12 cm pots containing 2 pinebark : 4 sand : 1 perlite amended with 2 $kg.m^{-3}$ low phosphorous Osmocote (18.0N:2.1P:9.0K). One seedling from each of 5 pots per population was transplanted into a tube giving a total of 55 seedlings for DNA analysis.

DNA analysis. DNA was extracted from 200-400 mg of seedling leaf tissue using a modified CTAB extraction method (Rogers and Bendich, 1985). Random amplified polymorphic DNA (RAPD) analysis was initially performed with 40 random primers from Operon Kits B and F. Thirty-six primers gave clear banding patterns so primers for the study were chosen arbitrarily from them.

Amplification of DNA was performed in 25ml reactions containing 67mM Tris-HCl (pH 8.8), $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.02% gelatin, 4mM $MgCl_2$, 200ml each dATP, dCTP, dGTP, dTTP, 0.25mM primer, 0.25mg genomic DNA and 1U *Taq* polymerase Stoffel fragment (Perkin Elmer). Thermal cycling was performed in a DNA thermal cycler (Corbett Research FTS-960) with the following profile: 5 min at 94°C (1 cycle); 1 min at 94°C, 1 min at 36°C, 2 min at 72°C (40 cycles); 3 min at 72°C. Amplification products were separated by electrophoresis of 1.5% agarose gel at 80v for 1.5 h in TAE buffer and visualised with ethidium bromide.

RESULTS

Mature floret morphology. In overall size, the mature florets of *A.adamsonii* are between the relatively small *A. aemula* and *A. billardierei* (Table 1). Results for *A.adamsonii* ranged from 1.6-3.5 mm (mean=2.3 mm) for overall lemma length, 1.0-4.2 mm (mean=2.4 mm) for awn length, 1.5-3.0 mm (mean=2.1 mm) for palea length and 0.4-2.4 mm (mean=1.4 mm) for rachilla extension length. The awns of *A.adamsonii* are significantly shorter than in all other species and are attached at a higher point on he lemma backs. the lemmas do not have long setae points, unlike *A aemula* and *A. billardierei*. Lemmas are variably hairy with some populations being completely glabrous (D, MMC and MT). Although all the *A.adamsonii* populations have significantly longer lemmas and paleas than *A. avenacea*, some of the populations with smaller floret dimensions have comparable rachilla extension lengths.

Variation between populations of *A.adamsonii* tended to be greater than variation within the one population (Table 1). For example, lemma length between populations had a variance of 0.764 compared to 0.0102 within populations. Awn length had a variance of 0.3240 between populations compared to 0.0140 within populations and the palea had a between population variance of 0.0712 compared to a within population variance of 0.0086.

DNA Analysis. This preliminary DNA study has shown that RAPD analysis enables differentiation of *A.adamsonii* from other *Agrostis* species and can provide information on genetic diversity. Due to the small number of plants analysed to date, insufficient information is available to compare genetic diversity or to define population genetic structure. Using primers OPB-08 and OPB-10, 24 bands could be scored. Individual banding patterns combining results from both primers were coded into haplotypes and the presence of haplotypes recorded for each population (Table 2). A total of 24 haplotypes occurred in the 41 plant analysed. The ability of RAPDs to differentiate between species was highly dependent on the primer used. Primer OPB-10 readily distinguished *A.adamsonii* from other *Agrostis* species but did not provide information on genetic diversity. Other species were more variable with OPB-10. Primer OPB-08 detected higher levels of diversity in all species tested but could not distinguish between species.

DISCUSSION

It is suspected that *A. adamsonii* has always been rare but its similarity to *A. billardierei, A. avenacea* and *A. aemula* which grow in the same or adjacent ecosystems has also resulted in it being overlooked. Whilst only preliminary, the DNA data has been useful in confirming the separation of species based on morphological characters. Further information from additional primers and individuals in each population will enable a robust assessment of genetic diversity and population genetic structure. As choice of primer affects the level of diversity which is detected, additional primers need to be assessed for their ability to

produce clear banding patterns for all the species under study. One primer has been found which can separate *A. adamsonii* from other species but it provides little information on genetic variation in *A. adamsonii*. It appears that the populations of *A.adamsonii* share a very similar gene pool although the populations are separated by several kilometres.

Information on the reproduction biology of *A. adamsonii* is not available although wind dispersal of pollen is probable. Seed dispersal between sites seems unlikely. A combination of molecular and morphological characters will probably be the most reliable for determining taxonomic delimitations and genetic structure and diversity of populations. Additional molecular work needs to be complemented with information on the breeding system of *A.adamsonii* so that sites can be managed to take into account the reproductive requirements of the species.

CONCLUSION

Both morphological and molecular data contribute to the taxonomic and genetic understanding of *Agrostis* species. RAPD analysis has given useful preliminary information will be used for a more comprehensive study of the taxon.

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Table 1

Comparison of mean mature floret characteristics for each population and for individual plants of smallest (D) and largest (ESN) populations of *A. adamsonii*.

Popn	Species	lemma body, mm	lemma setae, mm	back hairs	side hairs	awn, mm	palea, mm	rachilla, mm	awn attach, %
WM	*A. avenacea* (weeping)	1.6*a*	<0.1*a*	2.0*c*	3.0*d*	4.9*e*	1.4*a*	0.9*a*	60*d*
SHR	*A. avenacea* (small, erect)	1.7*a*	<0.1*a*	2.9*c*	3.3*e*	6.1*g*	1.5*b*	1.1*b*	52*c*
D	*A. adamsonii*	2.0*b*	0.1*a*	0.0*a*	0.0*a*	2.2*b*	1.8*c*	1.1*b*	76*f*
MMC	*A. adamsonii*	2.2*c*	<0.1*a*	0.0*a*	0.0*a*	2.1*b*	2.0*d*	1.4*c*	75*f*
HF	*A. adamsonii*	2.2*c*	<0.1*a*	0.9*b*	2.0*c*	1.6*a*	2.0*d*	1.0*ab*	84*g*
HSA	*A. adamsonii*	2.2*c*	<0.1*a*	0.0*a*	0.2*a*	2.5*c*	2.0*d*	1.4*c*	76*f*
MT	*A. adamsonii*	2.4*d*	<0.1*a*	0.0*a*	0.0*a*	2.4*bc*	2.1*e*	1.4*c*	77*f*
ESN	*A. adamsonii*	2.8*e*	0.1*a*	0.8*b*	1.5*b*	3.4*d*	2.6*g*	1.9*d*	69*e*
EM	*A. aemula* var. *aemula*	2.7*e*	1.0*d*	3.0*d*	3.3*e*	9.8*l*	2.5*f*	2.5*f*	39*b*
HSB	*A. billardierei* var. *robusta*	2.8*e*	0.7*b*	0.0*a*	0.0*a*	5.7*f*	2.6g	2.2*e*	55*c*
HD	*A. billardierei* var. *filifolia*	3.4*f*	0.8*c*	0.0*a*	0.0*a*	8.4*g*	3.5*h*	2.9*g*	24*a*

Popn	Plant	lemma body, mm	lemma setae mm	back hairs	side hairs	awn, mm	palea, mm	rachilla, mm	awn attach, %
D	1	2.0*a a*	0.1*a*	0.0*a*	0.0*a*	2.1*a*	1.9*b*	0.8*a*	79*b*
D	2	1.9 *a*	0.1*a*	0.0*a*	0.0*a*	1.9*a*	1.6*a*	1.0*a*	76*ab*
D	3	2.1 *a*	<0.1*a*	0.0*a*	0.0*a*	2.3*a*	1.8*ab*	1.2*a*	74*ab*
D	4	2.0 *a*	0.1*a*	0.0*a*	0.0*a*	2.2*a*	1.9*b*	1.2*a*	77*ab*
D	5	2.1 *a*	<0.1*a*	0.0*a*	0.0*a*	2.2*a*	1.8*ab*	1.2*a*	72*a*

Popn		lemma body,	lemma setae, mm	back hairs mm	side hairs	awn, mm	palea, mm	rachilla, mm	awn attach, %
ESN	1	2.9 *bc*	0.1*a*	0.8*a*	1.2*ab*	3.7*a*	2.6*a*	2.1*bc*	75*c*
ESN	2	2.4 *a*	0.1*a*	0.4*a*	1.6*b*	3.3*a*	2.4*a*	1.8*ab*	76*c*
ESN	3	3.0 *c*	0.1*a*	1.6*b*	2.2*c*	3.3*a*	2.6*a*	2.0*bc*	65*ab*
ESN	4	3.0 *c*	<0.1*a*	0.8*a*	1.4*ab*	3.2*a*	2.9*b*	2.3*c*	61*a*
ESN	5	2.7 *b*	<0.1*a*	0.6*a*	1.0*a*	3.2*a*	2.5*a*	1.5*a*	71*bc*

Note: lemma back and side hairiness was scored on a 0-4 (zero to very hairy) index
awn attach = attachment from lemma base as % of total lemma length

Figure 1
Distribution of *A. adamsonii* in Australia.

Table 2

Frequency of haplotypes in each *Agrostis* population and band presence/absence comprising each haplotype (bands scored for OPB-10 in italics, OPB-08 in standard font)

Popn	Species	Haplotype (frequency)
WM	*A. avenacea* (weeping)	X (0.80)W (0.20)
SHR	*A. avenacea* (small, erect)	V (1.0)
D	*A. adamsonii*	no data
MMC	*A. adamsonii*	F (0.40) H (0.40) G (0.20)
HF	*A. adamsonii*	B (0.75) C (0.25)
HSA	*A. adamsonii*	D (0.67) E (0.33)
MT	*A. adamsonii*	A (1.0)
ESN	*A. adamsonii*	J (0.50) I (0.25) K (0.25)
EM	*A. aemula* var. *aemula*	S (0.33) T (0.33) U (0.33)
HSB	*A. billardierei* var. *robusta*	L (0.60) M (0.20) N (0.20)
HD	*A. billardierei* var. *filifolia*	Q (0.40) O (0.20) P (0.20) R (0.20)

Haplotype	Band presence/absence
A	*1111000000000* 01011011010
B	*1111000000000* 01011111100
C	*1111000000000* 0111111100
D	*1111000000000* 01010111010
E	*1111000000000* 11010111000
F	*1111000000000* 00110011000
G	*1111000000000* 01110011000
H	*1111000000000* 01111011000
I	*1111000000000* 01111001000
J	*1111100000000* 01101001000
K	*1111000000000* 11101001000
L	*1001011000000* 01010100100
M	*1000011000000* 01010000110
N	*1001011000000* 01010100110
O	*1000011100001* 01010010000
P	*1000011100001* 01011010010
Q	*1000011100001* 01010010000
R	*1000011100001* 01011010000
S	*1000011010000* 01010100000
T	*1001001000000* 00010000100
U	*1011010010100* 00010000010
V	*1001011000000* 00110101010
W	*1000010000000* 00001010000
X	*1000010000001* 01010010000