Biochemical genetic markers to identify hybrids between *Aloe arborescens* and *A. ferox* (Aloaceae)

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Received 19 March 1996; revised 5 September 1996

Two populations of *Aloe arborescens*, six *A. ferox* populations, and a population of natural hybrids between *A. arborescens* and *A. ferox* were examined by horizontal starch gel-electrophoresis to assess levels of genetic variation and differentiation at 23 enzyme coding loci. Gene products revealed polymorphism in all of the populations studied, except for the four western populations of *A. ferox*. This indicates that the latter are founder populations from few parents, displaying only the dominant alleles, and with a possible migration route from east to west (as was also found for other plant taxa in South Africa). Biochemical genetic markers to identify *A. arborescens* × *A. ferox* hybrids were found at the DDH-2 and MNR-2 enzyme coding loci. Unique alleles were found in pure *A. arborescens* and *A. ferox* populations at these loci, which is an important result because it was not always possible to identify individuals correctly from their morphological characters. Genetic distance (Nei 1978) values between conspecific populations ranged from zero, between the western *A. ferox* populations, to 0.007 (*A. arborescens*) and to 0.01 (*A. ferox*, eastern populations), and it averaged 0.174 between the pure species.

Keywords: Allozyme variation, *Aloe arborescens*, *A. ferox*, electrophoresis, genetic distance, hybrids.

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Introduction

Aloe ferox Mill. forms the basis of a multimillion rand industry and the dried leaf exudate, known as Cape aloes, is used in several traditional medicines. Substantial quantities are exported to Europe and the leaf gel has also found a use in the production of cosmetic products. *Aloe ferox* and *A. candelabrum* Berger are described as morphologically similar species and occupy a taxonomic position in *Aloe* series Pachydendron (Reynolds 1982). A recent biochemical and chemical (leaf exudate) study confirmed *A. ferox* to be conspecific with *A. candelabrum* (Viljoen *et al.* 1996). In addition, Stokes and Yeaton (1995) and Hoffman (1988) showed that insect pollination is negligible in both *A. candelabrum* and *A. ferox*, and that birds are the only effective pollinator guilds. Initial studies on the genetic variation in *A. ferox* (van der Bank *et al.* 1995) revealed remarkably low levels of genetic variation throughout the wide distribution range of this species.

Studies of leaf exudate chemistry (Viljoen *et al.* in prep.) have concluded that hybridization has played a very important role in the evolution of *Aloe*, which complicates and masks many of the patterns which may have existed prior to hybridization events. As hybridization may defy any chemical or morphological detection, a study of natural hybrids between *A. ferox* and *A. arborescens* was considered necessary to establish the feasibility of horizontal starch gel-electrophoresis in detecting hybridization in the genus *Aloe*. The remarkable lack of reproductive isolation amongst relatively unrelated species such as *A. ferox* and *A. arborescens* also deserves further study, as it may help explain the lack of character congruence at the infrageneric level.

Materials and Methods

Leaf samples from distant populations of *A. arborescens*, hybrid individuals, and six natural populations of *A. ferox* were collected in liquid nitrogen (Table 1; Figure 1). The populations of *A. arborescens* and *A. ferox* were chosen because they are geographically isolated from each other so there is no possibility of hybridization or introgression and also because they represent the full range of morphological and chemical variation within the species. *Aloe ferox* populations from the Umtamvuna Valley (F1 & F2) were selected since they display the highest levels of terrain and morphological diversity when compared to other populations. This area represents an assemblage of reproductive and vegetative characters suggested to be diagnostic for *A. ferox* (recently synonymized with *A. candelabrum*), i.e. erect leaves for the former, white-lipped petals for the latter. The approximate distances between these populations are: Stormvleikloof to Riversdale *ca.* 107 km, Riversdale to Perseverance *ca.* 388 km, Perseverance to Jansenville *ca.* 142 km, Jansenville to Oribi Flats *ca.* 550 km, and Oribi Flats to Ixopo *ca.* 60 km.

Natural hybrids between *A. arborescens* and *A. ferox* from the southern part of the Western Cape are accurately described in Rey-

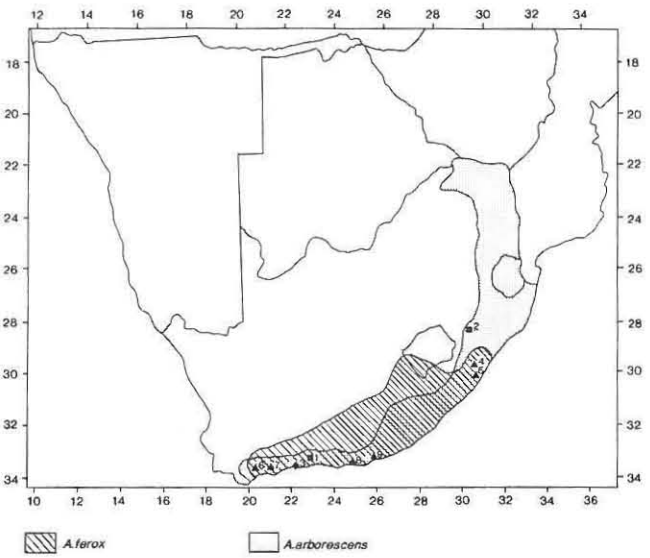

Figure 1 Natural distribution in South Africa and collection sites of *Aloe arborescens* (■): 1 = Montagu Pass, 2 = Estcourt; 3 = *A. arborescens* × *A. ferox* (◆), and *A. ferox* (▲): 4 = Ixopo, 5 = Oribi Flats, 6 = Stormsvleikloof, 7 = Riversdale, 8 = Jansenville and 9 = Perseverance.

Table 1 Localities and sample size of *Aloe* populations and species studied

Species	Population	Locality	*n*	Abbreviation
A. arborescens:	1. Montagu Pass	33 22 CD	5	A1
	2. Estcourt	29 29 BB	5	A2
A. arborescens × *A. ferox*:	3. Herbertsdale	34 21 BB	17	F×A
A. ferox:	4. Ixopo	30 30 AA	25	F1
	5. Oribi Flats	30 30 CA	25	F2
	6. Stormvleikloof	34 20 AA	55	F3
	7. Riversdale	34 21 AB	25	F4
	3. Jansenville	32 24 DC	25	F5
	9. Perseverance	33 25 DC	25	F6

*Populations are numbered as in Figure 1

nolds (1982) as: 'Plants are branched and have tall stems with persistent old dry leaves. The rosettes have large gracefully spreading leaves and branched inflorescence with large scarlet racemes.' Our sample contained individuals looking like typical *A. arborescens*, typical *A. ferox*, and hybrid individuals (as described above).

Collection, tissue preparation, extraction buffers, electrophoresis, staining of gels, interpretation of results, locus nomenclature and statistical analysis follow van der Bank *et al.* (1995). Gel and electrode buffers (Table 2) are described by Kephart (1990), and DISPAN (Copyright: Tatsua Ota, 1993, Pennsylvania State University, USA) is used to construct a phylogenetic tree using Nei's (1978) genetic distance, neighbour-joining and bootstrap tests (1 000 replications).

Table 2 Locus abbreviations, enzyme commission numbers (E.C. no.) and buffers systems used are listed after each enzyme

Enzyme (loci)	E.C. no.	Buffer	pH
Aspartate aminotransferase (AAT-1,-2)	2.7.3.2	PO	8.7
Cytosol aminopeptidase (CAP)	3.4.11.1	AA	8.6
Dihydrolipoamide dehydrogenase (DDH-1,-2)	1.8.1.4	HC	5.7
Esterase (EST-1,-2)	3.1.1.-	HC	5.7
Glucose-6-phosphate isomerase *(GPI-1,-2)	3.5.1.9	MF	8.6
Isocitrate dehydrogenase *(IDH-1,-2,-3)	1.1.1.42	HC	6.5
Malate dehydrogenase *(MDH-1,-2,-3)	1.1.1.37	HC	5.7
Menadione reductase *(MNR-1)	1.6.99.-	AA	8.6
(MNR-2)		AA	8.6
		HC	6.5
Phosphoglucomutase (PGM)	5.4.2.2	MF	8.6
		HC	5.7
6-Phosphogluconate dehydrogenase (PGD)	1.1.1.44	HC	6.5
Peroxidase *(PER-1,-2)	1.11.1.7	AA	8.6
Shikimate dehydrogenase (SKDH)	1.1.1.25	HC	6.5
Superoxide dismutase *(SOD)	1.15.1.1	MF	8.6

*Monomorphic loci; AA: tris–EDTA–borate; HC: histidine–citrate; MF: tris–EDTA–borate; PO: tris–citrate

Results

Twenty-three protein coding loci provided interpretable results in all *Aloe* populations analysed, and these data could be used for comparative studies and to calculate the extent of differentiation between populations. Twelve of the loci (52.2%) displayed monomorphic gel banding patterns (Table 2) in all populations, and isozyme differences occurred at 11 loci (Table 3). Markers were found at DDH-1 and MNR-2, where the hybrid had intermediate allele mobilities at the former locus and shared the alleles at the latter locus with the pure species (Figure 2).

Average heterozygosity (H) values, mean number of alleles per locus (A) and percentage of polymorphic loci (P) are listed in

Table 3 Allele frequencies for polymorphic loci (see Table 1 for population designations)

		Population					
Locus	Allele	F1	F2	F3–6	F×A	A1	A2
AAT-1	*A*	0.075*	0.063*		0.038*		0.333
	B	0.825	0.708	1.000	0.731	0.750*	0.333
	C	0.100	0.229		0.231	0.250	0.333
AAT-2	*A*	0.094	0.214			0.125	
	B	0.906	0.786	1.000	1.000	0.875	1.000
CAP	*A*	0.262	0.083		0.067*		
	B	0.738	0.917	1.000	0.933	1.000	1.000
DDH-1	*A*	1.000	1.000	1.000	0.625		
	B				0.375	1.000	1.000
DDH-2	*A*	1.000	1.000	1.000			
	B				1.000		
	C					1.000	1.000
EST-1	*A*	0.412	0.423		0.500		
	B	0.588	0.577	1.000	0.500	1.000	1.000
EST-2	*A*	0.568*	0.636*	1.000	0.611	0.500	0.750
	B	0.432	0.364		0.389	0.500	0.250
MNR-2	*A*	1.000	1.000	1.000	0.500*		
	B						0.167*
	C				0.500	1.000	0.833
PGM-2	*A*	0.182*	0.109				
	B	0.818	0.891	1.000	1.000	1.000	1.000
PGD	*A*	0.375					
	B	0.375	0.250*	1.000	0.500*	0.750	1.000
	C	0.250	0.750		0.250	0.250	
	D				0.250		
SKDH	*A*	0.348*	0.114*		0.393*		0.333*
	B	0.652	0.886	1.000	0.607	1.000	0.667

*Significant ($P < 0.05$) deviations of allele frequencies from expected Hardy–Weinberg proportions

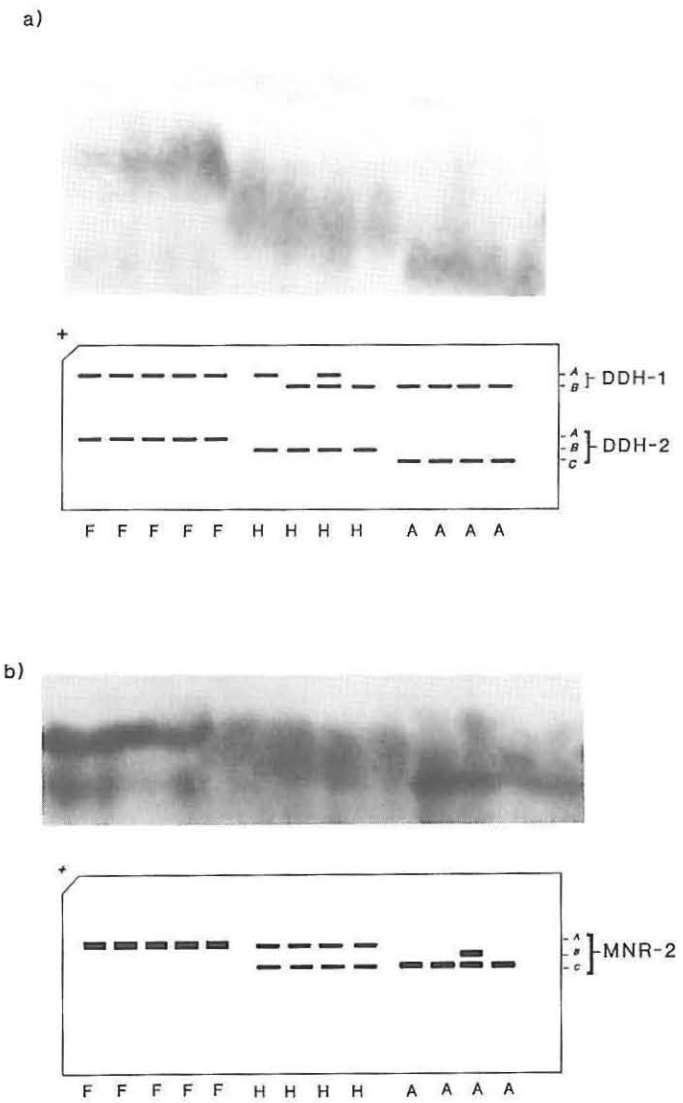

Figure 2 Zymograms showing (a) the alternative (DDH-1) and intermediate (DDH-2) positions of allele mobilities, and (b) shared mobilities at MNR-2 for the *A. arborescens* × *A. ferox* hybrids using histidine–citrate buffers with pH of 5.7 and 6.5 respectively (F = *A. ferox*, H = hybrid, A = *A. arborescens*).

Table 4. The *H* values were lower in *A. ferox* (0-0.148) and *A. arborescens* (0.074–0.078), and higher in the hybrid population (0.168). Other genetic variation parameters (*P* and *A*) showed a similar trend (Table 4).

Genetic distance (Nei 1972) values ranged from 0–0.02 between conspecific populations and it averaged 0.183 between the pure species studied. These values were 0–0.01 and 0.174 respectively for Nei's (1978) genetic distance (Table 5). Figure 3 shows the relationships between taxa, with the position of the hybrid intermediate compared with that of the pure species.

Discussion

Patterns of intraspecific variation often provide insight into evolutionary forces operating in populations. In the present study, we found genetic markers (Figure 2) to identify hybrids. The hybrids exhibited character coherence by sharing the species-specific alleles of the pure species. Molecular markers provide a powerful means to study introgressive hybridization, and unlike morphological characters, it possesses simple modes of expression and inheritance (Gottlieb 1981). The character association of allozygous gene expression by the hybrids, as shown in Figure 2, is a typical phenomenon in plant and animal species (e.g. van Vuuren *et al.* 1989; Nason *et al.* 1992; Sezaki *et al.* 1994; Riesenberg 1995). From the MNR-2 locus shown in Figure 2, it is obvious that the hybrids share the alleles of the parent species, with half the intensity of that of the parents. It is clear that these plants do not represent heterozygous individuals, since the relative mobility of the alternate (*B*) allele of the *A. arborescens* heterozygote (second individual from the right-hand side of the gel) is less than that of the *A*-allele. The intermediate position of the allele mobilities for the hybrid is also evident in Figure 2a. These biochemical markers are valuable to identify the hybrids in the population studied (which were sometimes impossible to identify from their morphological characters).

It is evident that the western (F3–6) populations of *A. ferox* have a depauperated genetic variation (Tables 2 & 3) compared with that of populations F2 and F1. The last population has the highest amount of variation ($H = 14.8\%$), followed by F2 (12%), and the other *A. ferox* populations (Table 4). This indicates that a south-western migration route was followed or that selective pressure was stronger towards the west, similar to the pattern obtained for *Virgilia oroboides* (van der Bank *et al.*1996).

Nei's (1972) measure was used by Thorpe (1982) to estimate genetic distance values between animal and plant taxa. Values of less than 0.3 are predicted for conspecific populations. We calculated genetic distances of 0–0.183 (Table 5) which are less than

Table 4 Average heterozygousity (*H*), mean number of alleles per locus (*A*), standard errors thereof, and percentage of loci polymorphic (*P*). See Table 1 for population designations

Population	*H* (S.E.)	*A* (S.E.)	*P*
F1	0.148 (± 0.048)	1.43 (± 0.14)	34.78
F2	0.120 (± 0.039)	1.39 (± 0.12)	34.78
F3–6	0.000 (± 0.000)	1.00 (± 0.00)	0.00
FxA	0.168 (± 0.053)	1.43 (± 0.14)	34.78
A1	0.074 (± 0.036)	1.17 (± 0.08)	17.39
A2	0.091 (± 0.045)	1.22 (± 0.11)	17.39

Table 5 Nei's (1972) genetic distance above diagonal and Nei's (1978) genetic distance below diagonal. See Table 1 for population designations

Population	F1	F2	F3–6	F×A	A1	A2
F1	*****	0.016	0.040	0.085	0.188	0.196
F2	0.010 (± 0.007)	*****	0.046	0.090	0.182	0.204
F3–6	0.037 (± 0.016)	0.044 (± 0.027)	*****	0.106	0.165	0.164
FxA	0.074 (± 0.056)	0.080 (± 0.056)	0.099 (± 0.054)	*****	0.109	0.112
A1	0.179 (± 0.094)	0.174 (± 0.093)	0.159 (± 0.087)	0.097 (± 0.058)	*****	0.020
A2	0.184 (± 0.092)	0.193 (± 0.095)	0.157 (± 0.084)	0.097 (± 0.059)	0.007 (± 0.006)	*****

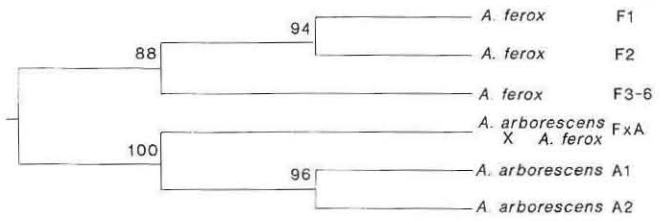

Figure 3 Dendrogram of phenetic relationships between taxa studied. Bootstrap numbers are listed at the nodes and population designations are presented in Table 1.

the above-mentioned range, and fall within the range for conspecific populations. Nei's (1978) genetic distance is better suited for small sample sizes (e.g. for the *A. arborescens* populations), however, the values are similar for both of Nei's (1972,1978) indices (Table 5). Figure 3 shows the relationships between the populations studied, with the hybrid in an intermediate position, as expected.

In conclusion, it is evident that the hybrid and both parent species can be identified by genetic markers. Our study is the first to show that enzyme electrophoresis provides a valuable tool for identifying hybrid aloes, particularly when morphological characters are ambiguous.

Acknowledgements

We thank SASOL for providing financial support to complete this study.

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