A chemotaxonomic and biochemical evaluation of the identity of *Aloe candelabrum (Aloaceae)*

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Summary

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In situ morphological analysis of plants from the entire distribution area supports the assumption that *Aloe candelabrum*, by its reproductive and vegetative characters, falls well within the taxonomic concept of *A. ferox*. The identical chemical composition of leaf exudate with regard to secondary compounds (chromone and anthrone derivatives) supports this conclusion. Gene products at 23 enzyme-coding loci, analyzed by horizontal starch gel-electrophoresis, revealed no fixed allele differences between *A. candelabrum* and *A. ferox*. The differences on which the two species were previously separated can be explained as local variation within a single and widespread taxon. *A. candelabrum* should therefore be subsumed under *A. ferox*.

Introduction

Aloe candelabrum A. Berger is described by Reynolds (1950) as a striking and stately species of *A.* sect. *Pachydendron* Haw. Its similarity with *A. ferox* Mill. has, however, been a topic of dispute and source of confusion. Reynolds (1950), Jeppe (1969), and Bornman & Hardy (1971) have invoked certain diagnostic characters in their attempts at making the two taxa mutually exclusive. Results of a comparison of the morphology, leaf exudate chemistry and allozyme variation throughout the natural distribution areas are reported in this paper. Our aim was a critical evaluation of the currently accepted hypothesis that *A. candelabrum* and *A. ferox* are two geographically vicarious species.

Material & methods

Morphological variation in populations of *Aloe ferox* and *A. candelabrum* was compared in their wild habitat during two successive flowering seasons (southern hemisphere winters of 1993 and 1994). Together the field trips covered almost the entire geographical distribution range of the two taxa (Fig. 1). During these surveys particular attention was paid to the diagnostic characters used by Reynolds (1950). For the purpose of this investigation the pressing of plant material was considered to be unnecessary, as all localities are precisely defined.

Leaf exudate samples were collected from three to seven individuals in each of 41 populations, resulting in a total of 169 samples (Fig. 1, Table 1). As all samples were collected in July, seasonal variation is eliminated as a possible distorting factor. The fresh exudate was air-dried and investigated by thin layer chromatography (TLC) and

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Table 1. Mean values of major leaf exudate compounds in 26 populations of *Aloe ferox* and 15 populations of *A. candelabrum* (expressed as percentage of total yield as estimated from HPLC results). Major compounds are numbered as in Fig. 2 and 3. The lowest and highest population means for *A. ferox* (see Van Wyk & al., 1995) and the means for individual populations of *A. candelabrum* are given.

Population No.	Locality	Sample No.	Major compounds 1	2	3	4	5	6	7 & 8
A. ferox									
1 to 26	range over entire distribution	76	25.8-32.4	2.2-8.4	34.9-52.3	2.8-7.7	5.2-15.4	4.8-16.2	0.0-3.4
A. candelabrum									
27	Umtamvuna River valley	3	24.0	0.0	45.0	5.9	5.4	6.5	0.0
28a	Oribi Gorge	3	30.5	0.6	41.9	3.9	4.2	5.0	0.0
28b	15 km on road to Oribi Flats	3	30.1	0.0	45.0	6.3	3.7	4.7	0.0
28c	7 km on road to Oribo Flats	3	32.0	0.0	42.1	5.1	3.7	4.4	0.0
29a	11 km northwest of iZingolweni	3	29.0	0.0	44.0	4.9	3.9	4.3	0.0
29b	17 km northwest of iZingolweni	3	24.9	0.0	33.2	8.2	8.9	10.6	0.0
29c	31 km northwest of iZingolweni	3	23.2	0.0	42.2	5.1	7.0	8.5	0.0
30	11 km north of uMzimkhulu	3	20.3	1.2	31.4	5.9	12.3	14.9	0.0
31a	21 km north of Ixopo	3	26.4	1.1	35.0	6.2	6.0	7.1	0.0
31b	28 km north of Ixopo	3	25.6	0.6	29.5	4.8	4.2	5.3	0.0
31c	33 km north of Ixopo	3	18.6	1.7	28.5	4.9	7.2	8.7	0.0
32	Otto's Bluff	3	17.8	1.0	35.4	1.6	10.8	14.6	0.0
33a	Ashburton	3	21.6	0.6	37.8	4.7	6.9	8.9	0.0
33b	Inchanga	3	26.8	1.5	36.1	5.0	4.9	5.8	0.0
33c	40 km east of Pietermaritzburg	3	25.4	0.0	38.7	7.3	5.7	6.4	0.0

high pressure liquid chromatography (HPLC). Samples were dissolved in methanol and passed through C_{18} cartridges to remove substances of high retention time. The purified samples were dissolved in methanol-water (1 : 1) and injected into the HPLC system. The system comprised a Phenomenex IB-Sil column (C_{18} reverse phase, 5 µm particle size, 250 mm × 4.6 mm internal diameter) with a flow rate of 1 ml min^{-1}, a 20 µl sample loop and a 30-60 % linear gradient of methanol in water over 25 min, 3 min isocratic, 100 % in 2 min, 4 min isocratic. For general screening this system was adjusted (40-80 % in 12 min, 80-100 % in 1 min, isocratic for 4 min). Detection was by diode array detector, using two channels (A set at 275 ± 70 nm; B at 365 ± 40 nm). TLC was carried out on silica gel (Merck) plates using the solvent system ethylacetate-methanol-water (100 : 16.5 : 13.5) as eluent. Compounds were identified by comparisons of Rf-values, visibility and colour under UV (254 nm and 366 nm), retention times and UV/VIS spectra with authentic reference samples (see Van Wyk & al., 1995).

For electrophoretical analysis, leaf samples from six distant populations were collected (Table 2; Fig. 1). Populations that are geographically isolated from each other were chosen, so that there is no possibility of hybridisation or introgression and so as to represent the full range of morphological and chemical variation within the species. *Aloe candelabrum* populations from Ixopo (population 31) and the Umtam-

Table 2. Localities and sample size (n) of *Aloe* populations and species used for enzyme electrophoresis.

Species	Locality	n	Population
A. candelabrum	Ixopo	25	31
	Oribi Flats	25	28
A. ferox	Stormvleikloof	55	1
	Riversdale	25	2
	Jansenville	25	11
	Perseverance	25	16

vuna Valley (population 28) were selected since they display the highest levels of morphological diversity. Plants growing in this area present a blend of reproductive and vegetative characters suggested to be diagnostic for both *A. candelabrum* (e.g. white-lipped petals) and *A. ferox* (e.g. erect leaves). The approximate distances between these populations and genuine *A. ferox* populations are: Stormvleikloof to Riversdale, c. 107 km; Riversdale to Perseverance, c. 388 km; Perseverance to Jansenville, c. 142 km; Jansenville to Oribi Flats, c. 550 km; and Oribi Flats to Ixopo, c. 60 km.

Collection, tissue preparation, extraction buffers, electrophoresis, staining of gels, interpretation of results, locus nomenclature and statistical analysis follow Van der

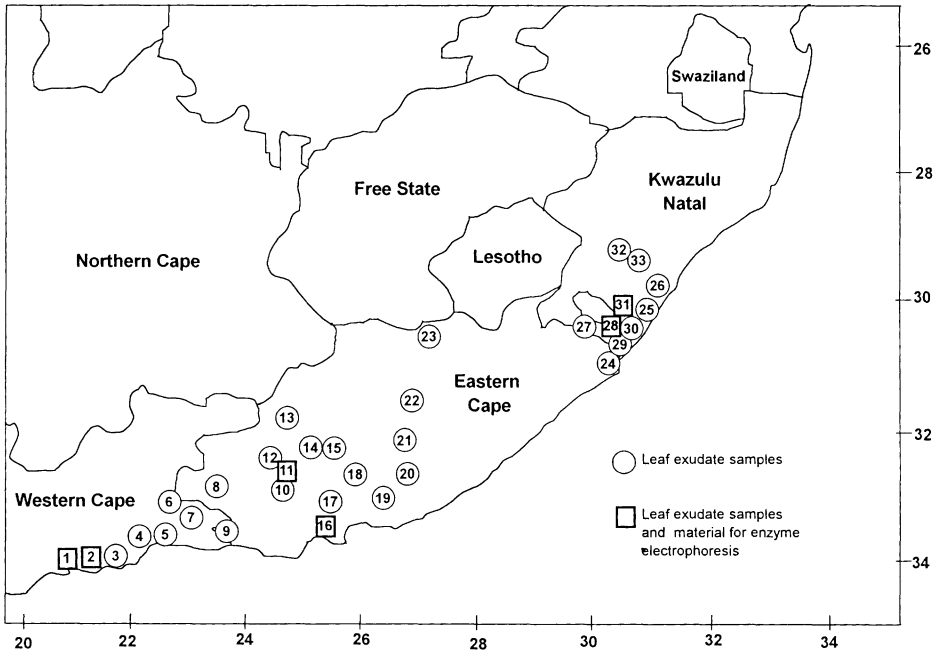

Fig. 1. The geographical distribution of *Aloe ferox* and *A. candelabrum* combined, with circles denoting the sampled populations (*A. ferox:* 1-26; *A. candelabrum:* 27-33), and squares denoting the sources of enzyme samples (*A. ferox:* 1, 2, 11 & 16; *A. candelabrum:* 28, 31). Locality details are given in Tables 1 and 2.

Table 3. Allozyme analyses performed on 6 populations (see Table 2) of *Aloe ferox* and *A. candelabrum*. Locus abbreviations, enzyme commission numbers (E.C. No.) and buffer systems used are listed after each enzyme. * = Monomorphic loci. – AA: Tris-EDTA-Borate; HC: Histidine-Citrate; MF: Tris-EDTA-Borate; PO: Tris-Citrate.

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, -2)*	1.6.99.–	AA	8.6
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

Table 4. Summary of the main morphological distinctions between *Aloe ferox* and *A. candelabrum*, as given by Reynolds (1950: 463, 468, 469).

Species	Rosette	Leaf disposition and length	Upper leaf face	Terminal raceme	Inner perianth segment apices
A. ferox	smaller than in *A. candelabrum*	densly crowded; shorter than in *A. candelabrum*	flat near base, canaliculate upwards; smooth to spiny	as long as lateral ones	brown to deep brown
A. candelabrum	larger than in *A. ferox*	spreading to recurved; longer than in *A. ferox*	rather deeply channelled; smooth	longer than lateral ones	clear white

Bank & al. (1995b). Gel and electrode buffers (Table 3) were as described by Kephart (1990). Wright's (1978) fixation indexes were also calculated: F_{IS} for individuals relative to the total population; F_{IT} for the total population and its subpopulations and F_{ST} for the amount of differentiation among subpopulations relative to the limiting amount under complete fixation.

Results and discussion

Morphology. – Reynolds (1950: 465, 470) discussed the supposed reproductive and vegetative morphological differences between *Aloe ferox* and *A. candelabrum* in his benchmark publication on *Aloe,* as listed in Table 4. Unfortunately some of these

differences are weak and not fully diagnostic, e.g. leaf length. Also, our own observations are often at variance with those of Reynolds. For example, plants growing close to the Umtamvuna River at the eastern border of the E. Cape Province (our population 27) have the apex of the inner perigone segments distinctly white, even

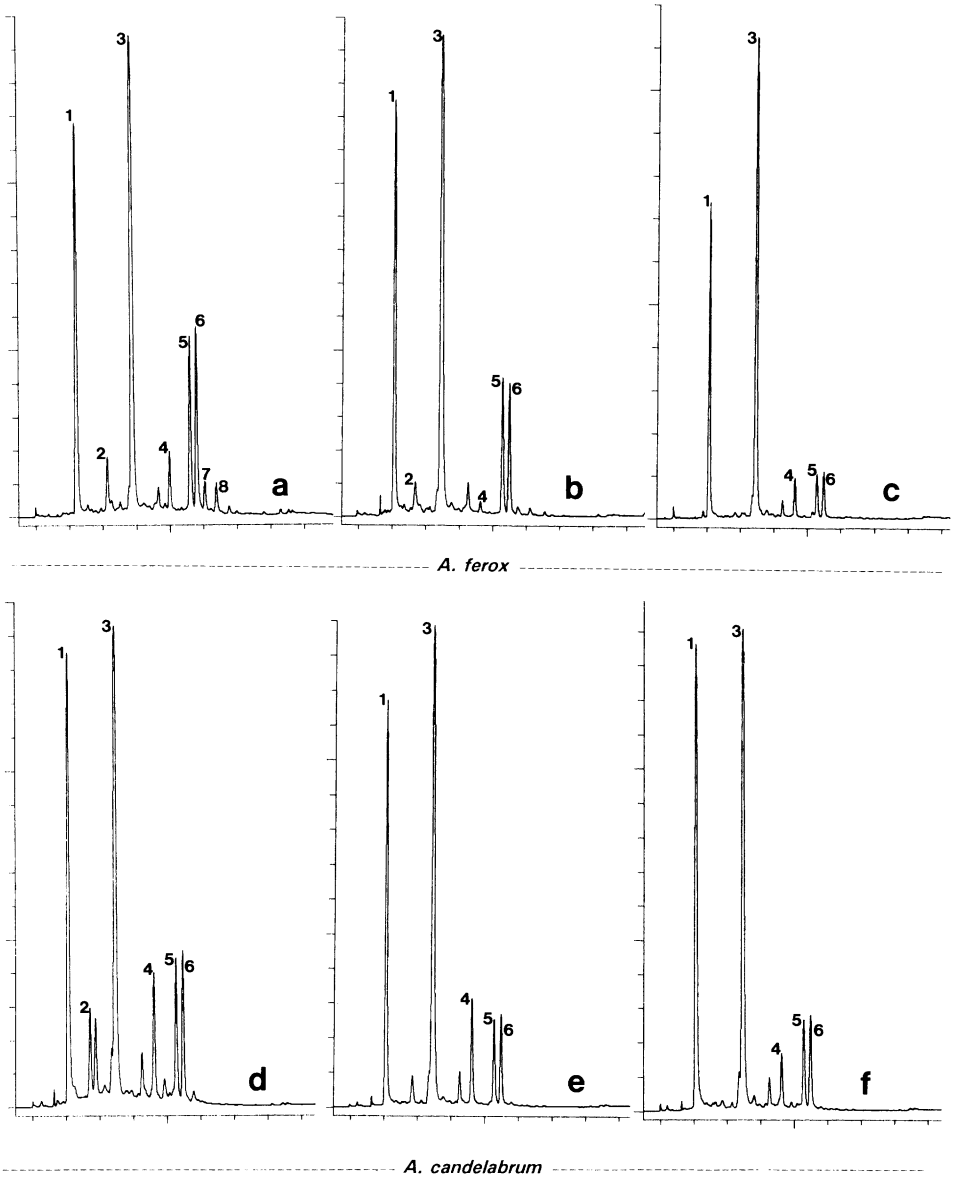

Fig. 2. Examples of typical HPLC chromatograms of the leaf exudate compounds of *Aloe candelabrum* and *A. ferox*, illustrating the range of variation in major chromone and anthrone derivatives (numbered as in Fig. 3.). Provenance of the samples: a, 3.5 km E of Heidelberg; b, Umtamvuna River; c, Robinson Pass; d, Oribi Gorge (population 28a); e, 28 km N of Ixopo (31b); f, 31 km NW of iZingolweni (29c).

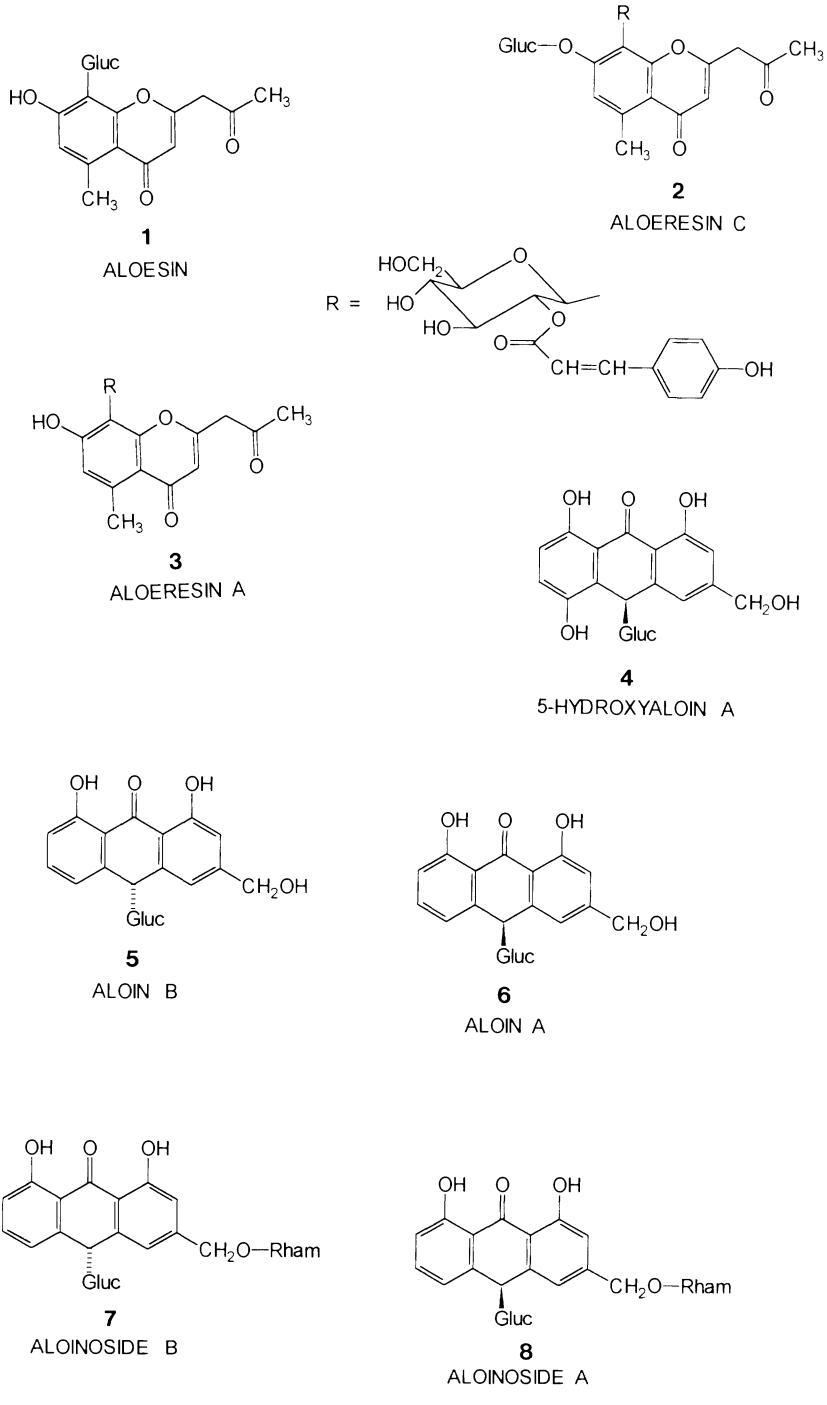

Fig. 3. Strutural formulae of chromone (1-3) and anthrone derivatives (4-8) found in *Aloe candelabrum* and *A. ferox*.

though the plants that occur in this area fall into Reynolds's concept of *A. ferox,* in which the apex of the inner perigone segments is supposed to be brown to deep brown (Table 4). In the Umtamvuna region, as in several other localities, the terminal racemes are sporadically taller than the lateral ones, a character state supposedly diagnostic for *A. candelabrum*. In all characters listed in Table 4, *A. ferox* is an extremely variable species across its entire distributional range, which stretches over more than 1000 km (Fig. 1). The differences between *A. ferox* and *A. candelabrum* as enumerated by Reynolds (1950) are merely of degree, indicating that upholding both as separate species is not justified.

Leaf exudate chemistry. – The geographical variation of *Aloe ferox* leaf exudate chemistry is detailed in Van Wyk & al. (1995). The composition of major chromone and anthrone derivatives was found to vary remarkably little. The main components characteristic of *A. ferox* (the fresh leaf exudate as well as the commercial dry product known as Cape Aloes) are aloesin, aloeresin A, aloeresin C, 5-hydroxyaloin A, and two isomers each of aloin and its rhamnosides (Hörhammer & al., 1963 & 1965; Van Rheede van Oudtshoorn & Gerritsma, 1964; Gramatica & al., 1982; Speranza & al., 1985; Rauwald & Beil, 1993a). Aloesin, aloin A, aloin B, and 5-hydroxyaloin A have been reported from *A. candelabrum* (McCarthy, 1969; Rauwald & Beil, 1993b). The characteristic chemical profile of *A. ferox* is repeated in *A. candelabrum* in all populations investigated (Table 1). Fig. 2 and 4 allow for a visual assessment of the quantitative similarity in the composition of compounds.

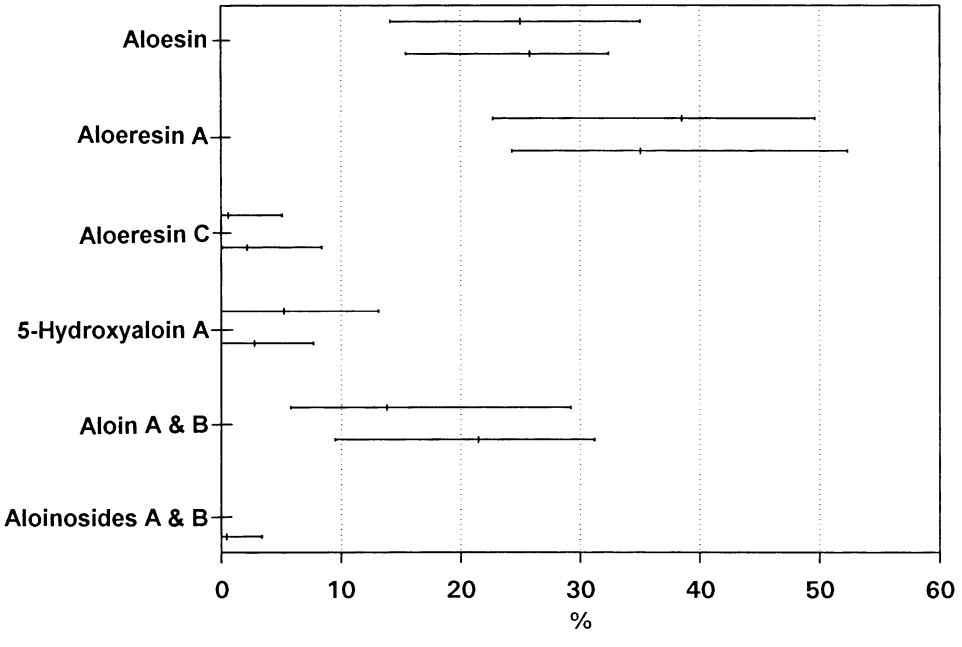

Fig. 4. A comparison of the range and mean values of estimated percent of total yield for each of the major leaf exudate compounds in *Aloe candelabrum* (upper bars; 45 samples) and *A. ferox* (lower bars; 76 samples). The aloinosides are found only in some populations of *A. ferox*, mainly in the extreme western parts of the distribution area.

Table 5. Results of allozyme analyses, performed on 6 populations (see Table 2) of *Aloe candelabrum* (28, 31) and *A. ferox* (1, 2, 11, & 16). Allele frequencies for polymorphic loci, fixation indices, average heterozygosity (*H*), mean number of alleles per locus (*A*), standard errors thereof, and percentage of loci polymorphic (*P*). * = Significant ($P < 0.05$) deviations of allele frequencies from expected Hardy-Weinberg proportions.

Locus	Allele	Population			Wright's (1978) *F*-statistics		
		31	28	1, 2, 11 & 16	F_{IS}	F_{IT}	F_{ST}
AAT-1	A	0.075*	0.063*	–	0.631	0.664	0.089
	B	0.825	0.708	1.000			
	C	0.100	0.229	–			
AAT-2	A	0.094	0.214	–	–0.028	0.058	0.083
	B	0.906	0.786	1.000			
CAP	A	0.262	0.083	–	0.250	0.338	0.117
	B	0.738	0.917	1.000			
EST-1	A	0.412	0.423	–	0.121	0.290	0.193
	B	0.588	0.577	1.000			
EST-2	A	0.568*	0.636*	1.000	0.571	0.650	0.184
	B	0.432	0.364	–			
PGM	A	0.182*	0.109	–	0.372	0.412	0.064
	B	0.818	0.891	1.000			
PGD	A	0.375		–	0.758	0.856	0.407
	B	0.375	0.250*	1.000			
	C	0.250	0.750	–			
SKDH	A	0.348*	0.114*	–	0.798	0.830	0.116
	B	0.652	0.886	1.000			
H (SE)		0.148 ± 0.048	0.120 ± 0.039	0.00 ± 0.000			
A (SE)		1.43 ± 0.14	1.39 ± 0.12	1.00 ± 0.00			
P		34.78	34.78	0.00			

The range of variation in each of the major constituents is remarkably similar. The apparent difference in mean aloin concentrations does not reflect the close agreement of the eastern populations of *A. ferox* with *A. candelabrum*. Although chemical similarity in itself is insufficient evidence for combining taxa, the important point here is that *A. ferox* and *A. candelabrum* are the only two taxa of *A.* sect. *Pachydendron* which are chemically identical. Furthermore, none of the other species of the section has the characteristic composition of chromone and anthrone derivatives that is shown in Fig. 2.

Allozymes. – 23 protein coding loci provided interpretable results in all *Aloe* populations analyzed, and these data could be used for comparative studies and to calculate the extent of differentiation between populations. 15 of the loci (65.2 %)

displayed monomorphic gel banding patterns (Table 3) in all populations, and allozyme variation occurred at 8 loci (Table 5). Fixed allele differences were not found in any of the populations studied, and the *A. ferox* populations (1, 2, 11 and 16) displayed the dominant alleles also found in the *A. candelabrum* populations. Average heterozygosity *(H)* values, mean number of alleles per locus *(A)* and percentage of loci polymorphic *(P)* are also listed in Table 5. The *H* values were zero in *A. ferox,* 0.12 in population 28 and as high as 0.148 in population 31 *(A. candelabrum)*. Other genetic variation parameters *(P* and *A)* showed a similar trend as for *H,* with an increase in variation from the south-western to north-eastern regions of the country (Table 5).

Deviations of allele frequencies from expected Hardy-Weinberg proportions occurred at none (1, 2, 11 and 16), four (28) and five (31) of the loci studied (Table 5). Since they were not observed at all loci, non-random mating, gene flow and genetic drift were probably not the factors responsible for these deviations, since these processes should affect all loci equally (Soltis & Soltis, 1988). Selection may likely have contributed to the above result. Nevertheless, it is evident that the western (1, 2, 11 and 16) populations of *A. ferox* show less genetic variation (Table 5) than those of *A. candelabrum* (28 and 31). Population 31 has the highest rate of variation (H = 14.8 %), followed by population 28 (H = 12 %), and the *A. ferox* populations (H = 0 %). This, together with the fact that the latter populations (1, 2, 11 and 16) display only the dominant alleles (Table 5), indicates that *A. ferox* may have expanded south-westwardly to conquer its present area.

Wright's (1978) fixation index quantifies inter-population differentiation. The mean F_{ST} value (0.203) for polymorphic loci (Table 5) in the aloes studied indicates a low amount of genetic differentiation between the populations, resulting from genetic drift. The extent of allelic fixation of individuals as compared to their subpopulation (F_{IS} = 0.465) also reflects genetic drift. Values of F_{IS} are close to zero in most natural populations where random mating within subpopulations occur (Nei, 1986). The F_{IT} value of 0.574 (which quantifies inbreeding due to population subdivision) is not indicative of effective barriers to gene flow between the populations studied. This is in agreement with geographical data (no geographical discontinuities exists between the populations studied).

Genetic distance (Nei, 1978) values ranged from 0.01 between *Aloe candelabrum* populations (28 and 31) to 0.04 between them and the *A. ferox* populations. Similarly, Nei's (1972) genetic distance values were 0.016 between populations 28 and 31, 0.040 between population 31 and *A. ferox,* and 0.046 between population 28 and *A. ferox*.

Nei's (1972) measure was used by Thorpe (1982) to estimate genetic distance values among animal and plant taxa. Values of less than 0.3 were found to be predictive for conspecific populations. Our calculated maximum genetic distance, 0.046, is lesser by almost an order of magnitude than the upper limit given by Thorpe (1982) for populations of the same species. However, Nei's (1978) genetic distance is better suited for small sample sizes. The values, which are similar for both of Nei's (1972, 1978) indices, strongly indicate that the observed genetic differentiation pertains to conspecific populations.

No biochemical differences were found to distinguish *Aloe candelabrum* from *A. ferox*. The suggested conspecificity is also substantiated by the distributional patterns (no geographical gap separates these taxa from each other) and by overlap in

morphological characters. We also reported high amounts of genetic variation in populations 28 and 31 (Table 5). The average *H* value of 4.5 % for all six populations studied is concordant with expected values for natural plant taxa (see Van der Bank & al., 1995a). This contradicts the original hypothesis by Van der Bank & al. (1995b) to explain the low amount of genetic variation obtained in populations 1, 2, 11 and 16.

Conclusion

No single morphological, chemical or biochemical character, or character combination, could be found to support the division of *Aloe ferox* into more than one species. In view of this lack of diagnostic characters, we conclude that *A. candelabrum* is conspecific with *A. ferox,* the name that takes precedence. We therefore propose the following synonymy (for a complete list of synonyms, see Reynolds, 1950: 460-462):

Aloe ferox Mill., Gard. Dict., ed. 8: *Aloe* No. 22. 1768.
= *Aloe candelabrum* A. Berger, Notizbl. Königl. Bot. Gart. Mus. Berlin 4: 246-247. 1906.

Acknowledgements

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