Pergamon

0305-1978(95)0007-0

*Biochemical Systematics and Ecology*, Vol. 23, No. 3, pp. 251–256, 1995

0305-1978/95 $9.50+0.00

# Genetic Variation in Two Economically Important *Aloe* Species (Aloaceae)

HERMAN VAN DER BANK,* BEN-ERIK VAN WYK† and MICHELLE VAN DER BANK‡

*Research Unit for Aquatic and Terrestrial Ecosystems, Rand Afrikaans University, P.O. Box 524, Auckland Park, Johannesburg 2006, South Africa;
†Department of Botany, Rand Afrikaans University, P.O. Box 524, Auckland Park 2006, South Africa;
‡Department of Botany, University of the Witwatersrand, Johannesburg 2050, South Africa

**Key Word Index**—*Aloe ferox*; *Aloe marlothii*; allozyme variation; isozyme differences; electrophoresis; geographical variation; leaf exudates; genetic distance.

**Abstract**—Four morphologically distinct and geographically separated populations of *Aloe ferox* and one population of *A. marlothii* were examined by horizontal starch gel electrophoresis to assess levels of genetic variation at 22 protein coding loci. Leaf extracts were surveyed for 26 proteins; gene products revealed no polymorphism in any of the populations studied in the former species, and genetic variation at one locus (4.55%) in the latter species. The percentage of polymorphic loci was 4.76%; a value of 1.05 (±0.05) was obtained for the mean number of alleles per locus, and the average heterozygosity per locus was calculated at 0.022 (±0.022) for *A. marlothii*. We propose the ecological theory to explain the low levels of genetic variation obtained (i.e. species that are well adapted to their environment need less genetic variation). Isozyme differences between species were encountered at two loci to produce a mean genotypic distance index of 0.056, indicating a high degree of differentiation between species. This is unexpected since the two species studied are closely related and known to produce natural hybrids with *A. arborescens*. Biochemical markers could not be found to distinguish different chemovars of the two species. The remarkably low levels of genetic variation may be related to the xerophytic habit of the plants, perhaps making them less sensitive to drought stress as a selection pressure.

## Introduction

The Cape aloe (*A. ferox* Miller) has a wide distribution range of more than 1000 km (from Swellendam in the west to the Mtamvuna River in the east) and at most localities it is more variable between than within populations (Reynolds, 1982). These plants have a wide range of commercial and traditional uses, of which the production of the laxative drug, Cape aloes, is by far the most important. Minor uses include cosmetics (skin lotions and hair care products) and food (leaves are used for making jam). *Aloe marlothii* Berger is no longer used commercially for the production of drug aloes but it is important in traditional plant use. This includes the treatment of round-worm infections, using the powdered dry leaves in snuff, for stomach troubles, and for hastening weaning of children by rubbing the green leaf pulp over the breasts. *Aloe marlothii* has an even wider distribution than *A. ferox*, and within-population variation in shape, size and colour, as well as between-population variance is characteristic for this species (Reynolds, 1982). The two species provide an opportunity for a wider survey of plant taxa as proposed by Van der Bank *et al.* (1995), in which samples from various geographical regions and morphologically distinct populations are included.

The purpose of this study is to describe genetic variation and geographical relationships of morphologically different *A. ferox* populations, to determine the extent of genetic differentiation between populations and species (*A. ferox* and *A. marlothii*), to establish if biochemical markers exist for the identification of individuals containing aloin and homonataloin in the latter species, and to ascertain whether similarity

(*Received* 2 *November* 1994)

among populations at allozyme loci corresponds with relationships based on morphology and leaf exudate chemistry. A detailed account of the chemical variation in the two species will be published elsewhere.

## Materials and Methods

*Plant material.* Leaf samples from 50 *A. ferox* individuals each were collected from four natural populations (Table 1). Exudate samples were collected from all individuals. These populations were chosen because they are geographically isolated from each other so that there is no possibility of hybridisation or introgression, and also because they represent the full range of morphological and chemical variation within the species. Collection sites are shown in Fig. 1. The approximate distances between these populations are: Stormvleikloof–Riversdale *ca* 107 km, Riversdale–Perseverance *ca* 388 km and Perseverance–Jansenville *ca* 142 km. The *A. marlothii* population sampled from Pilansberg comprised 50 individuals, and it included both aloin- and homonataloin-bearing individuals. The exudate samples were collected as spots on filter paper (from the fifth leaf of each rosette), rapidly air-dried and analysed within a few days.

*Procedure.* The exudate was investigated on both TLC and HPLC. Samples were dissolved in methanol (TLC) and some selected samples of *A. ferox* (see Table 1) were then passed through C18 cartridges to remove substances of high retention time and redissolved in methanol–water (1:1) for HPLC injection. A Phenomenex IB-Sil column was used (C18 reverse phase, 5 μm particle size, 250 mm × 4.6 mm internal diameter; flow rate 1 ml min $^{-1}$; 20 μl sample loop; solvent system 30–60% linear gradient of methanol in water over 25 min, 3 min isocratic, 100% in 2 min, 4 min isocratic; diode array detector with two channels, A at 275 ±70 nm, B at 365 ±40 nm). For TLC we used silica gel (Merck) plates with ethylacetate–methanol–water (100:16.5:13.5) as eluent. Compounds were identified by comparison of *Rf* values, visibility and colour under UV 254 and 366 nm. The population of *A. marlothii* from Pilansberg was screened on TLC to identify aloin- and homonataloin-bearing individuals, but the exact proportions of major compounds were accurately quantified with HPLC for two individuals (Table 1).

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 Kepart (1990). The estimate of elapsed divergence time between species (Nei, 1987) was also calculated.

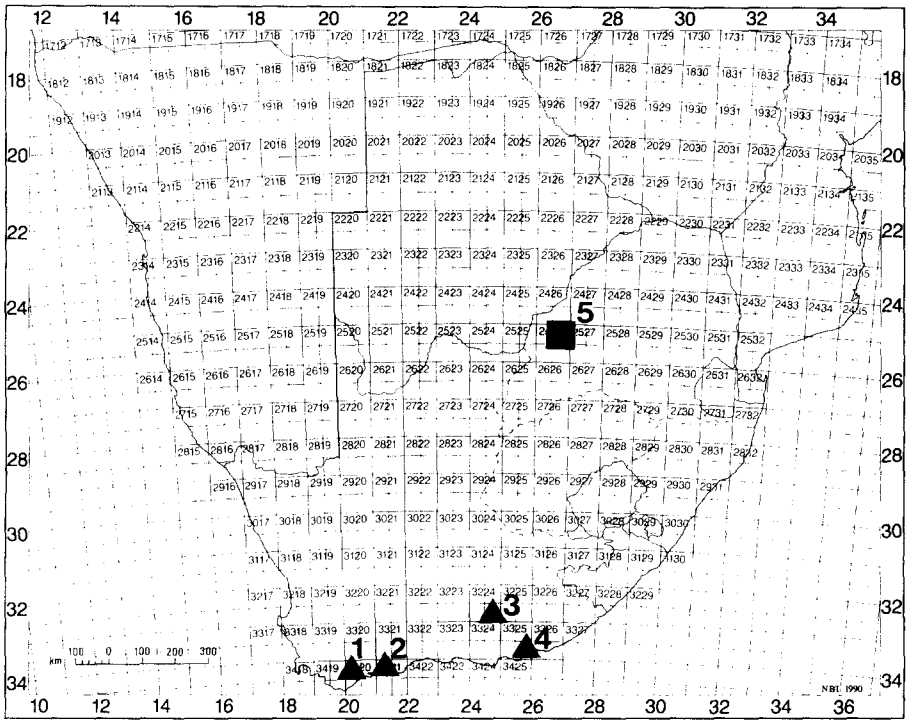


FIG. 1. COLLECTION SITES OF *ALOE FEROX* (▲): STORMSVLEIKLOOF (1) RIVERSDALE (2), JANSENVILLE (3) AND PERSEVERANCE (4); AND *A. MARLOTHII* (■, 5).

TABLE 1. LOCALITIES AND DESCRIPTIONS OF POPULATIONS OF *ALOE FEROX* AND *A. MARLOTHII* STUDIED, AND MAJOR LEAF EXUDATE COMPOUNDS IN PLANTS SELECTED FOR HPLC ANALYSIS

| Population | Major compounds (as % of total exudate) Aloesin | Aloe-resin A | Aloin* | Aloinoside* | Homonataloin* |
|---|---|---|---|---|---|
| *Aloe ferox:* | | | | | |
| Stormvleikloof (34°08'S, 20°06'E; tall, robust and uniform) | | | | | |
| Plant 1 | 17.3 | 25.7 | 28.3 | 9.0 | — |
| Plant 2 | 18.0 | 27.6 | 26.4 | 8.9 | — |
| Plant 3 | 18.6 | 24.2 | 26.0 | 8.5 | — |
| Riversdale (34°05'S, 21°16'E; short, variable, with small rosettes) | | | | | |
| Plant 1 | 17.8 | 23.8 | 27.4 | 8.0 | — |
| Plant 2 | 24.0 | 37.8 | 22.3 | 4.0 | — |
| Plant 3 | 21.2 | 35.7 | 26.1 | 5.9 | — |
| 7 km N of Jansenville (32°57'S, 24°41'E; tall, robust and uniform) | | | | | |
| Plant 1 | 21.4 | 24.3 | 13.7 | 1.8 | — |
| Plant 2 | 26.1 | 31.3 | 10.5 | 1.0 | — |
| Plant 3 | 40.3 | 23.4 | 16.0 | trace | — |
| Perseverance, near Port Elizabeth (33°59'S, 25°38'E; short, robust and variable) | | | | | |
| Plant 1 | 33.9 | 38.7 | 13.5 | trace | — |
| Plant 2 | 26.5 | 32.7 | 14.9 | trace | — |
| Plant 3 | 22.0 | 27.6 | 16.7 | trace | — |
| *Aloe marlothii:* | | | | | |
| Pilansberg (25°10'S, 26°56'E; variable) | | | | | |
| Plant 1 | 34.0 | 5.0 | — | — | 58.0 |
| Plant 2 | 37.0 | 20.0 | 22.0 | — | — |

*The two stereoisomers of both these compounds occur in more or less equal amounts and have been added together.

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 |
|---|---|---|---|
| Creatine kinase (*CK*) | 2.7.3.2 | **MC** | 6.1 |
| Dihydrolipoamide dehydrogenase (*DDH-1, -2*) | 1.8.1.4 | **HC** | 6.5 |
| Esterase (*EST-2*) | 3.1.1.- | **TEB** | 8.0 |
| Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH-2*) | 1.2.1.12 | **HC** | 5.7 |
| Glycerol-3-phosphate dehydrogenase (*GPD*) | 1.1.1.8 | **HC** | 5.7 |
| Glucose-6-phosphate isomerase (*GPI*) | 3.5.1.9 | **TEB** | 7.5 |
| Guanine deaminase (*GDA*) | 3.5.4.3 | **HC** | 5.7 |
| Hexokinase (*HK*) | 2.7.1.1 | **MC** | 6.1 |
| L-Iditol dehydrogenase (*SORD*) | 1.1.1.14 | **MC** | 6.1 |
| Isocitrate dehydrogenase (*IDH*) | 1.1.1.42 | **TEB** | 7.0 |
| L-Lactate-dehydrogenase (*LDH*) | 1.1.1.27 | **LB** | 8.3 |
| Malate dehydrogenase (*MDH-1, -2*) | 1.1.1.37 | **HC** | 6.5 |
| Malic enzyme (*ME-1, -2*) | 1.1.1.38 | **HC** | 5.7 |
| Mannose-6-phosphate isomerase (*MPI*) | 5.3.1.8 | **MC** | 6.1 |
| Menadione reductase (*MEN*) | 1.6.99.- | **HC** | 6.5 |
| Phosphoglucomutase (*PGM-1, -2*) | 5.4.2.2 | **HC** | 5.7 |
| 6-Phosphogluconate dehydrogenase (*PGD*) | 1.1.1.44 | **HC** | 5.7 |
| Purine-nucleoside phosphorylase (*NP*) | 2.4.2.1 | **MC** | 6.1 |

**HC**—Histidine–citrate; **LC**—lithium–borate; **MC**—morpholine–citrate; **TEB**—Tris–EDTA–borate.

## Results

Chemical variation in major leaf exudate compounds in the four populations of *A. ferox* and one population of *A. marlothii* is summarised in Table 1. Five individuals of *A. marlothii* contained aloin and 45 had homonataloin, but biochemical markers associated with this chemical dichotomy could not be found.

Twenty-two 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. Nineteen of the loci (86.4%) displayed monomorphic gel banding patterns in all populations, and isozyme differences occurred at two loci (*ME-1* was not present in *A. ferox* and *PGM-1* was not present in *A. marlothii*). The allelic products of the following loci migrated cathodally: *EST-2, GAPDH-2, GDA, HK, ME-2, MPI, NP, PGD, PGM-2* and *SORD.* Two loci were observed for *DDH,* and the enzymatic activity at the *EST-1* and *GAPDH-1* loci was not sufficient to score it satisfactorily.

Allelic frequencies ($fA = 0.64$; $fB = 0.36$) at the dimeric *MDH-1* locus did not deviate significantly ($P < 0.05$) from expected Hardy–Weinberg proportions ($\chi^2 = 0.87$; degree of freedom = 1) in *A. marlothii* and the individual heterozygosity value ($h$) was 0.461. Individuals with aloin and homonataloin were found to be homozygous at either of the alternate alleles or to be heterozygous at this locus. No polymorphism was obtained for any of the 200 *A. ferox* individuals studied.

We could not find sufficient activity when gels were stained for the following enzymes and proteins: acid phosphatase (E.C. 3.1.3.2), adenylate kinase (E.C. 2.7.4.3), aspartate aminotransferase (E.C. 2.6.1.1), general protein, peptidase (E.C. 3.4.-.-) using leucylglycylglycine, L-phenylalanyl–L-proline and leucyl–tyrosine as substrates, and peroxidase (E.C. 1.11.1.7). The mean number of alleles per locus ($A$) was one in all of the *A. ferox* populations studied and 1.05 in *A. marlothii.* The percentage of polymorphic loci was 4.76 and the average heterozygosity ($H$) value was 2.2% (±0.022) for the latter population. The genetic distance (Nei, 1972) value between species was 0.056 (±0.037) and the estimate of elapsed divergence time between species (Nei, 1987) is 0.28 million years ago.

## Discussion

Hardy–Weinberg proportions of allele frequencies were obtained at the only polymorphic locus found (*MDH-1*). This fact, together with the other alleles being fixed for single allele combinations (homozygotes), suggests that the species studied have stabilised and that the trend of low variability is unlikely to be due to the process of random genetic drift in this case. Selection for homozygote advantage is a more plausible explanation. Individuals with aloin and homonataloin possessed either of the alleles (or a combination thereof) at the *MDH-1* enzyme coding locus. This result, together with all the other loci being monomorphic, showed that no association could be found between electrophoretic and chemical results. The results from the present study also show that very little genetic variation could be found in the *Aloe* species studied when compared with that of other plant species. For example Van der Bank *et al.* (1995) obtained nearly an order of magnitude more genetic variation in *Virgilia oroboides* when allele frequencies at corresponding loci were analysed.

Genetic variation over populations is the result of various combinations of selection, mutation, migration, genetic drift and non-random mating. Combinations of these forces result in gene frequency distributions, which in turn result in distance values for populations from these distributions (Hendrick, 1975). Several factors may account for the lower allozyme variation obtained in the populations studied. Genetic bottlenecks associated with dispersal and subsequent establishment, or the gradual isolation of larger populations into smaller ones as a result of climatic changes or increasing fire frequencies, can cause low levels of heterozygosity in species. Founding of populations or increasing isolation of larger populations into smaller ones could deplete genetic variation by loss of alleles, and the generally small population sizes would keep diversity low (Crawford *et al.*, 1992). These factors are unlikely to be important in *A. ferox* and *A. marlothii,* two widespread species with considerable morphological variation within and between populations. *Aloe marlothii* in particular, is remarkably variable in its leaf exudate compounds, and several distinct chemovars can be distinguished on the basis of their anthrone components.

Another, more likely hypothesis was discussed by Lawson *et al.* (1989). According to this ecological theory, some organisms experience their environment as fine-grained, reflecting adaptation to a narrow niche, albeit with wide geographic ranges, within a stable environment. Such species will have less genetic variability than those experiencing their environment as coarse-grained. For example, crocodiles are the sole survivors of the Archosauria, which included the well-known and awe-inspiring dinosaurs. This fact, and also because these reptiles are extremely well-adapted to their environment, makes them good examples of fine-grained species. Lawson *et al.* (1989) reported $H$ values ranging from 0.9% to 3.4% (average = 1.8%) in various crocodilian populations and species. This trend of low variability in habitat specialists seems to support the hypothesis that environmental variables are the determinants of genetic variation. These values are in agreement with those obtained for the *Aloe* species we studied ($H$ = 0–2.2%). In addition, aloes are succulents thus making them better adapted to environmental extremes and are not edible, containing high levels of chromone and anthrone derivatives as anti-feedants. Based on the above facts, we propose that the aloes studied represent fine-grained species. This is substantiated by the low genetic variability obtained in the present study and the relatively old biogeographic age of aloes (Conran, in press), as also estimated from the time of elapsed divergence between species (0.28 million years ago). Furthermore, the xerophytic habit may make *Aloe* species relatively insensitive to normal selective forces driving genetic divergence in mesophytic taxa.

It is interesting that no genetic differentiation was obtained between populations of *A. ferox* (which showed morphological and chemical differences between populations and little differentiation within populations), but we did obtain some variation in *A. marlothii* (which displayed intrapopulation morphological variation). The morphological differences between populations may, therefore, be attributed to environmental influences in the former instance. Van Wyk *et al.* (1995) studied the geographical variation in the major compounds of *A. ferox* leaf exudate throughout its natural distribution range and found it to be remarkably invariable. This coincides with the genetic data, in that no differences were obtained within *A. ferox* populations, and that some variation was found in the heteromorphic *A. marlothii* population. In *A. marlothii*, the dispersal of various provenances (genotypes) may well have occurred, since some local inhabitants from southern Africa have the custom to plant an aloe from the home region of the deceased relative on the grave. This would also contribute in maximising gene flow between populations to the extent that overall geographical differentiation would decrease.

Despite morphological differences, our results indicate remarkably low genetic variation in the aloes studied. Judged by morphology and chemistry, *Aloe* species are no less variable than other genera and the lack of allozyme variation remains to be explained. It may be the result of technical limitations inherent in the method, or related to the successful modification to a succulent habit, which perhaps make these species relatively unaffected by normal selection pressures (of which drought stress is one of the most important factors in southern Africa). Since several generations were present at each of the sites sampled, it is unlikely that the limited genetic variation can be ascribed to disrupted gene flow from one population to the next, despite erratic regeneration in these long-lived species. It would be interesting to see if similar low levels of allozyme variation occur in other succulent groups. This lack of discrimination between populations of *A. ferox* have economic implications, because it will not be possible to distinguish between different provenances, so that enzyme electrophoresis appears to be unsuitable for genetic "fingerprinting". It seems that chemical analyses of the leaf exudate offer better possibilities for characterising different populations, as was done by van Wyk *et al.* (1995), who suggested that favourable chemotypes (populations with total aloin levels above 25%) should be selected.

Nevertheless, the discovery of isozyme differences between *A. ferox* and *A. marlothii*, two related species of the section *Pachydendron*, suggests that isozyme data may have value at higher taxonomic levels. For instance, it was recently suggested by Alvaro *et al.* (unpublished research, Rand Afrikaans University) that *A. candelabrum* be subsumed under *A. ferox*. It may also be interesting to study isozyme inheritance in hybrids, thereby contributing to a better understanding of their reproductive biology and providing a more objective measure for deciding on the circumscription of species.

**Acknowledgements**—We thank SASOL for providing financial support to complete this study and Dr G. F. Smith (NBI, Pretoria) for logistical assistance during sampling.

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