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Lack of genetic differentiation between 19 populations from seven taxa of *Sutherlandia* Tribe: Galegeae, Fabaceae

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Abstract

Horizontal starch gel-electrophoresis was used to examine genetic diversity between geographically isolated populations of *Sutherlandia tomentosa*, *S. frutescens*, *S. frutescens* var. *incana*, *S. microphylla*, *S. speciosa*, *S. humilis* and *S. montana* at 18 enzyme coding loci. *Astragalus atropilosulus* subsp. *burkeanus* and a *Lessertia* species were used as outgroups to root dendrograms. Gene product of 32 enzyme coding loci revealed genetic variation at 18 (56.3%) thereof. The percentage of polymorphic loci ranged from 0 to 20.6% (0.95 criterion), values between 1.0 ($\pm$ 0.00) and 1.3 ($\pm$ 0.09) were obtained for the mean number of alleles per locus, and average heterozygosity values per locus ranged from 0 to 0.097 ($\pm$ 0.034). The average genetic distance between populations (0.077) was higher than between taxa (0.032); the overlap in the cluster analyses of genetic distance data indicated that *Sutherlandia* taxa are not easily distinguished genetically. Although preliminary results showed that it was possible to identify some sympatric taxa, the analysis of additional populations annuled this result. There is remarkable lack of agreement between morphological and allozyme patterns within the genus *Sutherlandia* and the low allozyme differentiation between populations and taxa could be the result of the breeding systems of this genus. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Fabaceae; Cancer bush; *Sutherlandia*; Genetic variation; Enzyme electrophoresis

1. Introduction

The genus *Sutherlandia* R. Br., commonly known as the cancer bush, comprises six taxa, all of which are endemic to southern Africa (Phillips and Dyer, 1934). The

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taxonomically significant characters to distinguish the taxa are the habit, the orientation of the fruit stipe, the shape of the pods and the shape and pubescence of the leaflets. *Sutherlandia* taxa have both medicinal and horticultural uses. Leaf infusions of *S. frutescens* are used to treat stomach, intestinal and uterine ailments. It is also used as a cough remedy (Uphof, 1968), as a tonic (Van Wyk et al., 1997), to relieve eye troubles and chicken pox (Watt and Breyer-Brandwijk, 1962) and to treat internal cancers. The vernacular names refer to the use as a cancer cure, and there are several anecdotes to support this claim, but so far there is no published scientific evidence. Canavanine, a non- protein amino acid with known antitumourigenic properties, has been extracted from seeds of *S. frutescens* and further investigations seem worthwhile. The taxa were also grown as ornamentals in England as early as 1683 (Curtis, 1792).

Phillips and Dyer (1934) highlighted the problems associated with the taxonomy of this genus. It is still unclear whether there are six taxa, two, or perhaps only one. The taxonomy of *Sutherlandia* is problematic because the taxa often grade into each other (Phillips and Dyer, 1934). They can only be distinguished by a combination of characters and even this is not always conclusive (Schrire and Andrews, 1992).

Previous taxonomic research suggested that these taxa may be considered varieties of a large polymorphic taxon, and that the range of variation makes it difficult to separate the taxa (Meyer, 1836; Harvey, 1862; Schrire and Andrews, 1992). The existence of morphologically integrated populations also led Phillips and Dyer (1934) to identify the need for a genetic study of the genus *Sutherlandia*. There are no clear geographical patterns within taxa and each population has its own particular combination of morphological characters.

This paper is a first attempt to determine the extent of genetic differentiation in *Sutherlandia* and to evaluate the feasibility of using allozyme data as additional characters to morphology in grouping the populations and taxa.

2. Materials and methods

2.1. Plant material

Leaf samples of *Sutherlandia* individuals were collected from 19 natural populations: five populations of *S. frutescens* (L.) R. Br. ($N = 79$), two of *S. frutescens* var. *incana* E. Mey. ($N = 49$), two of *S. tomentosa* Eckl. and Zeyh. ($N = 76$), six of *S. microphylla* Burch. ex DC. ($N = 76$), two of *S. montana* Phill. and Dyer ($N = 40$), one of *S. humilis* R. Br. ($N = 20$), one of *S. speciosa* Phill. and Dyer ($N = 20$) and outgroups *Astragalus atropilosulus* (Hochst.) Burge subsp. *burkeanus* (Harv.) Gillett ($N = 2$) and a *Lessertia* sp. ($N = 5$). The latter is a new species, not yet described. Sample size for the ingroup taxa was subject to availability. *Sutherlandia* populations usually comprise a relatively small number of individuals growing in an area of less than 500 m^2. The populations are geographically separated and were chosen because they allowed comparisons of population, variety and taxon levels. They also represent the full range of morphological and geographical variation within the genus. Populations and taxa investigated, their sources and voucher specimens are denoted in Fig. 1

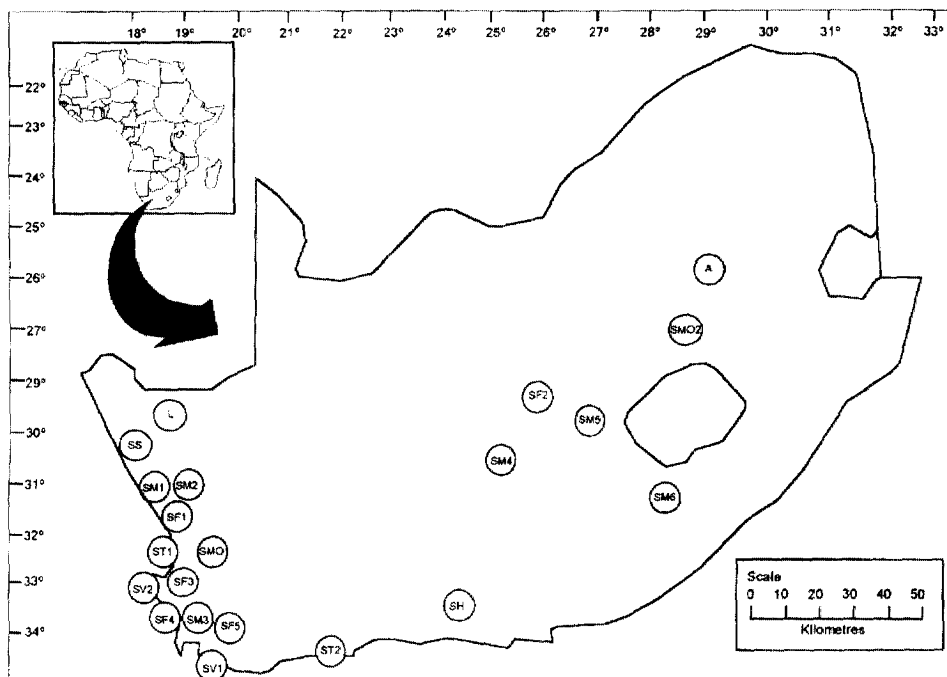

Fig. 1. Localities sampled for enzyme electrophoresis (populations are numbered as in Table 1).

and listed in Table 1. The identification of the populations is based on considerable field experience of the last ten years, using the diagnostic characteristics mentioned in the introduction.

2.2. Procedure

Young leaves were collected from growing shoots, placed in cryotubes and immediately submerged in liquid nitrogen (-196^0C). Sample preparation, extraction buffer, electrophoresis, staining of gels, interpretation of results, locus nomenclature and statistical analysis followed Van der Bank et al. (1995a). Locus abbreviations, monomorphic loci, buffer systems used and enzyme commission numbers are given in Table 2. Buffer systems used are described in Van der Bank et al. (1995a,b). The statistical analysis of allozyme data was carried out using BIOSYS-1 (Swofford and Selander, 1981). Allele frequency data for populations of the same taxa were combined and DISPAN (Ota, 1993) was used for the construction of dendrograms using the neighbour-joining algorithm. Bootstrap tests involved 1000 replications. Phylogenetic relationships were also investigated using Nei's (1978) genetic distance. Given the lack of published information regarding the isozymes and allozymes of the genus, we had to identify as many informative enzyme loci as possible. One population of *S. tomentosa* and two populations of *S. microphylla* were studied in a preliminary

Table 1
Source, location and number of individuals sampled per population of *Sutherlandia* studied

Taxa	Voucher specimens	Abbreviation	Source	GRID	*N*
Astragalus atropilosulus subsp. *burkeanus*	AdC 11	A	Randburg	2529 AC	1
Lessertia sp.	DM, B-EVW and AdC 6	L	Pofadder	2918 CB	5
S. frutescens	DM, B-EVW and AdC 14	SF1	Aurora	3318 DC	20
S. frutescens	DM, B-EVW and AdC 22	SF2	Fauresmith	2925 AC	20
S. frutescens	DM, B-EVW and AdC 15	SF3	Saldanha	3317 BC	5
S. frutescens	DM, B-EVW and AdC 5	SF4	Camps Bay	3318 CD	4
S. frutescens	DM, B-EVW and AdC 4	SF5	Worcester	3319 CC	19
S. frutescens var. *incana*	B-EVW 3668	SV1	Pearly Beach	3419 CB	20
S. frutescens var. *incana*	DM, B-EVW and AdC 16	SV2	Bloubergstrand	3318 CD	20
S. humilis	DM, B-EVW and AdC 19	SH	Uniondale	3323 CA	20
S. microphylla	DM, B-EVW and AdC 12	SM1	Vanrhynsdorp	3118 BC	20
S. microphylla	DM, B-EVW and AdC 13	SM2	Vanrhynsdorp	3118 BC	3
S. microphylla	DM, B-EVW and AdC 20	SM3	Leeuwberg Pas	3218 B	20
S. microphylla	B-EVW 3667	SM4	Colesberg	3024 DA	20
S. microphylla	B-EVW 3801	SM5	Tweeling	2927 CA	7
S. microphylla	B-EVW 3804	SM6	Elliot	3028 DD	7
S. montana	DM, B-EVW and AdC 10	SMO1	Piquetberg, De Hoek	3218 DD	20
S. montana	DM, B-EVW and AdC 23	SMO2	Reitz	2728 CD	20
S. speciosa	DM, B-EVW and AdC 8	SS	Khamiesberg	3017 BB	20
S. tomentosa	DM, B-EVW and AdC 1	ST1	Koeberg Nature Reserve	3118 AC	33
S. tomentosa	DM, B-EVW and AdC 6	ST2	Still Bay	3421 AD	43

Note: DM = Dineo Moshe, B-EVW = Ben-Erik van Wyk and AdC = Antonio de Castro.

Table 2
Locus abbreviations enzyme commission numbers (E.C. no.), buffers and their pH used in the study are listed after each enzyme

Enzyme	Locus	E.C. No	Buffer	pH
Adenylate kinase	*AK*	2.7.4.3	RW	8.0
Esterase	*EST-1,-2*	3.1.1.-	RW	8.0
	[a]*EST-3*			
Glyceraldehyde-3-phosphate dehydrogenase	[a]*GAPDH-1,-2*	1.2.1.12	HC	5.7
Glucose-6-phosphate isomerase	*GPI-1,-2*	3.5.1.9	RW	8.0
Isocitrate dehydrogenase	*IDH*	1.1.1.42	HC	6.5
Leucine aminopeptidase	[a]*LAP*	3.4.11.1	MF	8.6
Malate dehydrogenase	*MDH-1*	1.1.1.37	LiOH	8.1
	[a]*MDH-2,-3*			
Menadione reductase	[a]*MNR-1,-3*	1.6.99.-	MF	8.6
	MNR-2			
Manose-6-phosphate dehydrogenase	*MPI*	5.3.1.8	RW	8.0
Peptidase, substrate:				
Leucine-alanyl	[a]*PEP-C-1,-2*	3.4.-.-	MF	8.6
Leucylglycylglycine	[a]*PEP-B-1,-2,-3*			
Leucyl-tryosine	[a]*PEP-S-1,-2*			
Peroxidase	[a]*PER-1,-3*	1.11.1.7	MF	8.6
	PER-2			
6-Phophogluconate dehydrogenase	*PGDH-1,-2*	1.1.1.44	HC	6.5
Phosphoglucomutase	*PGM-1,-2*	5.4.2.2	MF	8.6
Superoxide dismutase	*SOD-1,-3*	1.15.1.1	RW	8.0

[a] Monomorphic loci.
MF: A continuous buffer (pH = 8.6) system (Markert and Faulhaber 1965).
RW: A discontinuous buffer (electrode pH = 8.0; gel pH = 8.7) system (Ridgway et al., 1970).
LiOH: A discontinuous buffer (electrode pH = 8.1; gel pH = 8.3) system (Kephart, 1990).
HC: A discontinuous buffer (electrode pH = 6.5; gel pH = 6.5) system (Kephart, 1990).

survey, and for these populations both monomorphic and polymorphic enzymes were identified. Only polymorphic enzymes were then analysed in subsequent studies. The results from both studies are reported in this manuscript.

3. Results and discussion

Thirty-two enzyme coding loci provided interpretable results in all populations analysed, of which 56.3% displayed polymorphism (Table 3). Fifteen of the 32 loci (43.7%) displayed monomorphic gel banding patterns and products of the *SOD-3* and *EST-3* loci migrated cathodally. In addition to these loci, the following enzymes were stained for: acid phosphatase (E.C. No. 3.1.3.2) and aspartate aminotransferase (E.C. No. 2.6.1.1). These two enzymes did not show sufficient activity or resolution to score them satisfactorily in *Sutherlandia* samples. At *SOD-2*, only a few taxa showed activity, hence the exclusion of this locus from Table 3. The maximum number of alleles for any

Table 3
Sample size and allele frequencies for polymorphic loci (see table 1 for taxa designation)

Locus	Taxa Allele	ST1 35	ST2 25	SV1 20	SV2 20	SM1 3	SM2 20	SM3 16	SM4 7	SM5 7	SM6 20	SF1 20	SF2 5	SF3 4	SF4 19	SF5 20	SMO1 20	SMO2 20	SH 20	SS 5	L 1	A 33
AK	A	1.000	0.986	0.980	1.000	1.000	0.833	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	0.972	1.000	1.000		1.000
	B		0.014	0.020			0.167					0.025						0.028			1.000	
EST-1	A	1.000	0.250	0.583	0.800	0.231	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					1.000	1.000		1.000
	B		0.750	0.417	0.200	0.769									1.000	1.000	1.000	1.000			1.000	
EST-2	A	1.000	0.632	0.812	1.000	1.000	1.000	1.000	1.000	0.857	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B		0.368	0.188						0.143												
GPI-1	A	1.000	0.141	0.948	0.316					1.000	1.000				1.000	1.000	0.800	0.800	0.611	0.316	0.500	
	B		0.859	0.052	0.684	1.000	1.000	0.975	1.000			0.974	1.000	1.000			0.100	0.200	0.389	0.579	0.500	
	C							0.025				0.026					0.100			0.105		1.000
GPI-2	A									1.000	1.000											1.000
	B		0.032																			
	C	1.000	0.968	1.000	1.000	0.667	0.800	0.778	1.000			1.000	0.333	1.000	1.000	1.000	1.000	1.000	0.824	0.500	1.000	
	D					0.333	0.200	0.222					0.667						0.176	0.500		
IDH	A																				1.000	
	B	1.000	1.000	1.000	1.000	0.750	1.000	1.000	1.000	0.143	0.143	1.000	1.000	1.000	1.000	0.053	0.700	0.833	0.824	0.176		
	C					0.250				0.857	0.857					0.947	0.300	0.167		0.824		1.000
	D																		0.176			
MDH-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B								1.000		1.000											
MNR-1	A											0.500										
	B	1.000	1.000	1.000	0.938	0.500	0.500	0.700	1.000	1.000	1.000	0.300	1.000	1.000	1.000	1.000	0.900	1.000	0.950		1.000	1.000
	C				0.062	0.500	0.500	0.300				0.200					0.100		0.050	0.053		
	D																			0.947		
MNR-2	A					1.000	1.000															
	B	1.000	0.949	0.964	1.000			0.917	0.976	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.833	1.000	1.000	1.000	1.000	1.000
	C		0.051	0.036				0.083	0.024								0.167					
MNR-3	A	1.000																				
	B		1.000	1.000	1.000	1.000	1.000	0.967	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.333	1.000
	C							0.033				0.025									0.667	

—continued

Table 3—*continued*

Locus	Taxa Allele	ST1 35	ST2 25	SV1 20	SV2 20	SM1 3	SM2 20	SM3 16	SM4 7	SM5 7	SM6 20	SF1 20	SF2 5	SF3 4	SF4 19	SF5 20	SMO1 20	SMO2 20	SH 20	SS 5	L 1	A 33
MPI	A	1.000	0.986	1.000	0.929	1.000	1.000	0.962	1.000	1.000	0.938	0.025	0.231	1.000	1.000		0.938	0.722	1.000	1.000	1.000	
	B		0.014		0.071			0.038			0.062	0.975	0.769			1.000	0.062	0.167				
	C																	0.111				1.000
PER-2	A		0.032																			
	B	1.000	0.968	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGDH-1	A	1.000	1.000	1.000	0.750	0.588		0.500				0.467	1.000	1.000	1.000	0.972	0.750	1.000	0.700	0.500		1.000
	B				0.250	0.412	1.000	0.500	1.000	1.000	1.000	0.533				0.028	0.250		0.300	0.500	1.000	
PGDH-2	A			0.967		0.125		0.267		0.143	0.143						0.025					
	B	1.000	0.974		1.000	0.875	1.000	0.733	0.684	0.857	0.714	1.000	1.000	1.000	0.500	0.889	0.525	1.000	0.812	0.656		
	C			0.033					0.316		0.143				0.500	0.111	0.450		0.188	0.344		
	D		0.026																		1.000	
	E																					1.000
PGM-1	A	1.000	0.949	1.000	0.200	0.386		0.322	0.884	0.286		0.079	0.094	1.000	1.000	1.000	0.750	0.667	0.750	0.650		
	B				0.650	0.478	0.833	0.571	0.058	0.571	1.000	0.763	0.812				0.250	0.278	0.225	0.225	1.000	
	C		0.051		0.150	0.136	0.167	0.107	0.058	0.143		0.158	0.094					0.055	0.025	0.125		
	D																					1.000
PGM-2	A	1.000	1.000	1.000	1.000	0.895	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	B					0.105																
SOD-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	B																					1.000
SOD-3	A																					1.000
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

given taxon was five and the average heterozygosity (*H*) values ranged between 0.01 and 0.097 (Table 4).

Astragalus atropilosulus subsp. *burkeanus* had unique alleles at *GPI-1*c* (Fig. 2a), *IDH*c*, *MPI*c*, *PGDH-2*e*, *PGM-1*d*, and high allele frequencies at *SOD-1*b* and *-3*a* as compared to *Sutherlandia* taxa, with A and B as alternate alleles at the latter two loci. The *Lessertia* species had a unique (A) allele at *IDH* whereas the *Sutherlandia* taxa had only BB and BC phenotypes at this locus.

3.1. *Genetic variation within Sutherlandia populations*

Of the 19 ingroup populations studied, five had unique alleles. For example, *S. microphylla* had unique alleles at *GPI-2*a* (SM5, SM6), *MDH-1*b* (SM4, SM6) and *MNR-2**a (SM1, SM2). *Astragalus atropilosulus* subsp. *burkeanus* shared *MNR-1*a* with SF1 and *MNR-3**a with ST1. The percentage of polymorphic loci (*P*) was the same for three populations, SM1, SH and SS (20.6), and varied from zero to 17.6 for the other populations; it was 8.8 for SF1, SF2, SV1, SM2 and SM6; 14.7 for SM3, SMO2 and ST2 and 5.9 for SF5, SM4 and L (Table 4).

Individual heterozygosity (*h*) values ranged from 0.05 to 0.6; the mean number of alleles per locus (*A*) was between 1.0 and 1.3 (Table 4); the observed number of heterozygotes (*OBS*) ranged from zero to 18 and the coefficient of heterozygosity deficiency (*d*) values were from − 0.029 to 0.900. Details regarding the observed number of heterozygotes, coefficients for heterozygosity deficiency or excess, X^2 values, degrees of freedom and individual heterozygosity values for individual loci can be obtained from the senior author (email: bot@rau3.rau.ac.za). Genotypic frequency deviations from expected Hardy-Weinberg proportions were detected at *EST-1* (SV2; SM1; SF1), *EST-2* (ST2; SM5), *GPI-1* (ST2; SS), *GPI-2* (ST2; SM1-3; SF2; SH; SS), *IDH* (SM1; SM5; SM6; SF5; SMO1-2; SH; SS), *MNR-1* (SS), *MNR-3* (L), *MPI* (SF2; SMO1-2), *PER-2* (ST2), *PGDH-1* (SV2; SM1; SM3; SF1; SF5; SMO1), *PGDH-2* (ST2; SM1; SM3; SM4; SM5; SM6; SF4; SF5; SMO1; SH; SS), *PGM-1* (SM1; SM3; SM4; SMO1-2; SH; SS) and *PGM-2* (SM1). Deficiencies of heterozygotes occurred at all these loci except for *MNR-1* (SS) and *PGM-1* (SM3). This could be due to small sample size and could not be corrected because the total population was sampled in most cases. The average heterozygosity (*H*) values per locus (Table 4) ranged from zero (A, SF3, ST) to 0.097 (SS); and variation at population level occurred at *GPI-1* between two populations of *S. microphylla* (SM5 and SM6) (Fig. 2b) and heterozygotes were present at *PGM-1* (Fig. 2c).

The values of population variability obtained in the present study (0–20.6%, 1–1.3, 0.01–0.097, respectively) are quite low as compared to the reported values for vascular plants in Hamrick and Godt (1983) ($P = 22.0$–75.3%, $A = 1.35$–2.56, $H = 0.079$-0.354). The lower allozyme variation between taxa might be attributed to various factors. For example, the breeding system is taxonomically important for three reasons: the extent of interbreeding largely defines the pattern of variation and hence the delimitation of taxa; a knowledge of the breeding system frequently helps to understand complexity, although often it does not solve the problems associated with it; and a study of the breeding system is often vital in unveiling evolutionary pathways

Table 4

Mean number of alleles per locus (A), percentage of polymorphic loci (P) using the 0.95 criterion, average observed heterozygosity per locus (H) and Nei's (1978) genetic distance values below diagonal. See Table 1. for taxa designation

Population	A	L	SF1	SF2	SF3	SF4	SF5	SV1	SV2	SH	SM1	SM2	SM3	SM4	SM5	SM6	SMO1	SMO2	SS	ST1	ST2
A	1.0	1.0	1.1	1.1	1.0	1.0	1.1	1.2	1.2	.1.2	1.2	1.1	1.3	1.1	1.1	1.1	1.2	1.2	1.3	1.0	0.0
	(± 0.0)	(± 0.02)	(± 0.09)	(± 0.07)	(± 0.0)	(± 0.03)	(± 0.05)	(± 0.01)	(± 0.02)	(± 0.02)	(± 0.09)	(± 0.05)	(± 0.09)	(± 0.07)	(± 0.07)	(± 0.07)	(± 0.09)	(± 0.09)	(± 0.03)	(± 0.0)	(± 0.00)
P	0	5.9	8.8	8.8	0	2.9	5.9	8.8	17.6	20.6	20.6	8.8	14.7	5.9	11.8	8.82	17.6	14.7	20.6	0	14.7
H	0.0	0.028	0.037	0.033	0.0	0.015	0.010	0.067	0.079	0.026	0.063	0.020	0.038	0.024	0.038	0.024	0.063	0.054	0.097	0.0	0.046
	(± 0.00)	(± 0.02)	(± 0.0)	(± 0.02)	(± 0.00)	(± 0.03)	(± 0.01)	(± 0.02)	(± 0.03)	(± 0.01)	(± 0.02)	(± 0.02)	(± 0.0)	(± 0.02)	(± 0.02)	(± 0.02)	(± 0.03)	(± 0.02)	(± 0.03)	(± 0.00)	(± 0.02)
A	–																				
L	0.515	–																			
SF1	0.437	0.156	–																		
SF2	0.373	0.227	0.048	–																	
SF3	0.391	0.221	0.086	0.054	–																
SF4	0.427	0.176	0.100	0.128	0.068	–															
SF5	0.431	0.214	0.058	0.099	0.090	0.094	–														
SV1	0.400	0.200	0.103	0.090	0.033	0.020	0.105	–													
SV2	0.384	0.158	0.043	0.032	0.019	0.067	0.095	0.040													
SH	0.360	0.174	0.084	0.053	0.017	0.043	0.105	0.014	0.043	–											
SM1	0.443	0.170	0.071	0.084	0.070	0.091	0.099	0.089	0.049	0.069	–										
SM2	0.475	0.176	0.094	0.087	0.089	0.166	0.188	0.126	0.051	0.074	0.034	–									
SM3	0.383	0.160	0.058	0.033	0.022	0.089	0.113	0.056	0.006	0.018	0.050	0.039	–								
SM4	0.472	0.209	0.116	0.119	0.064	0.128	0.158	0.101	0.066	0.060	0.109	0.087	0.051	–							
SM5	0.376	0.184	0.121	0.100	0.099	0.175	0.141	0.136	0.075	0.080	0.099	0.082	0.056	0.098	–						
SM6	0.080	0.210	0.152	0.131	0.153	0.227	0.192	0.194	0.112	0.127	0.142	0.115	0.094	0.080	0.034	–					
SMO1	0.413	0.137	0.072	0.110	0.067	0.005	0.070	0.027	0.052	0.038	0.070	0.133	0.070	0.110	0.128	0.174	–				
SMO2	0.410	0.160	0.057	0.087	0.056	0.013	0.061	0.021	0.042	0.040	0.062	0.129	0.068	0.125	0.142	0.191	0.106	–			
SS	0.359	0.178	0.112	0.073	0.052	0.091	0.094	0.065	0.094	0.028	0.080	0.091	0.039	0.081	0.047	0.092	0.065	0.081	–		
ST1	0.391	0.221	0.086	0.054	0.000	0.068	0.090	0.033	0.019	0.017	0.070	0.089	0.022	0.064	0.099	0.153	0.067	0.056	0.052	–	
ST2	0.422	0.187	0.064	0.078	0.021	0.035	0.065	0.027	0.030	0.034	0.054	0.111	0.044	0.089	0.120	0.179	0.034	0.024	0.073	0.021	–

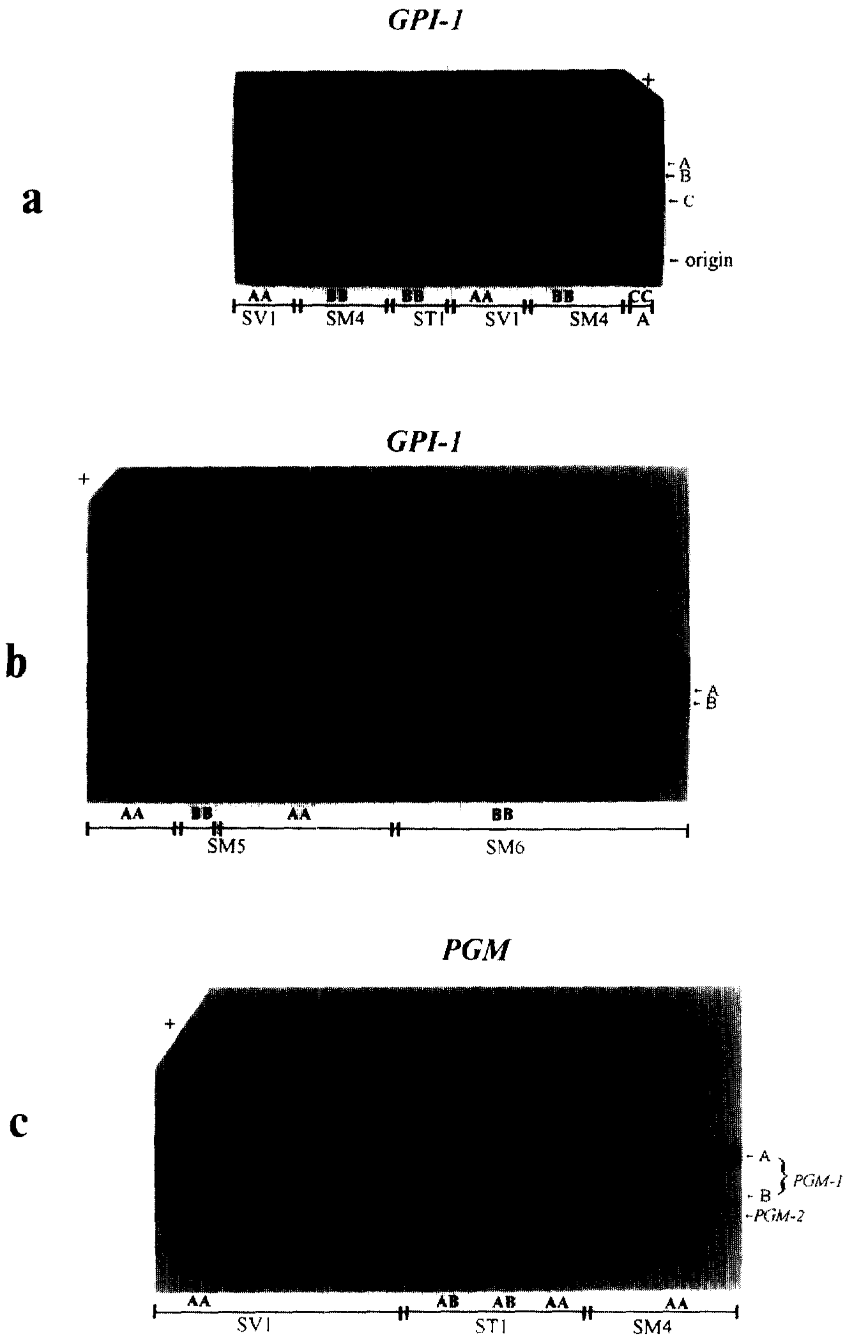

Fig. 2. An example of a gel showing allozyme variation at *GPI*-1 between (a) *Sutherlandia* taxa and (b) two *S. microphylla* populations, and (c) a gel showing allozyme variation at *PGM-1*.

(Stace, 1980). *Sutherlandia* taxa are pollinated by the malachite sunbird (*Nectarinia famosa*) commonly found in southern Africa (Skead, 1967; Arroyo, 1981). The bird can travel long distances and is able to transfer genetic material from different geographically isolated populations of taxa, causing the recombination of alleles. Flowers may also become cleistogamous, thus enabling self-pollination (B-EvW, pers.obs.). This will result in a small population becoming larger up to a point where pollinators will be attracted.

Cleistogamy would result in a morphologically uniform population which might later, during favourable seasons, be pollinated by a bird with pollen from a geographically distant population. The resulting seeds will have a combination of genetic material and this could result in high levels of integration and recombination. The high degree of integration among the taxa studied (Fig. 3b) makes it difficult to support the hypothesis that the genus comprises six taxa. The average D value between populations was 0.077 and a UPGMA dendrogram derived from D values shows the relationships and integration of 19 populations from six taxa of *Sutherlandia* and outgroups (Fig. 3b). The grouping of populations ST1 and SF3 was mainly due to lack of heterozygotes in both populations and had the highest bootstrap value (90). Most of the groupings of populations were not well supported as indicated by very low bootstrap values (Fig. 3b). It is interesting to note that all pairs of populations with relatively high bootstrap values (SM5 and SM6, SM1 and SM2, SV1 and ST2, and ST1 and SF3), respectively originated from the same geographical areas. This suggests that the geographical component of the allozyme pattern is somewhat stronger than the taxonomic component. A similar result was obtained by Hornero and Pèrez (1997) in *Colutea*.

3.2. Genetic differentiation

Using limited numbers of populations when investigating genetic variation may lead to incorrect conclusions about the taxa studied. In a preliminary survey, only seven *Sutherlandia* populations (SF4, SF5, ST1, ST2, SM4, SM5, SM6) were studied. Fig. 3a illustrates the relationship among the four taxa using *A. atropilosulus* subsp. *burkeanus* (A) as the outgroup. Except for *S. frutescens* all the taxa could be distinguished by unique alleles: *S. frutescens* var. *incana* (*PGDH-1*c*), *S. microphylla* (*MNR-2*c*, *PGDH-2*a* and *PGM-1*b*), *S. tomentosa* (*GPI-2*a* and *PER-2*a*) and *A. atropilosulus* subsp. *burkeanus* (*IDH*c*, *MDH-1*b*, *MNR-1*a*, *MPI*c*, *PGDH-2*e*, *PGM-1*d*, *PGM-2*b* and *SOD-1*b*). From this cluster three groupings were evident and the grouping of taxa agreed with the classification based on morphology. The cophenetic index value was 99.9%. We then investigated more populations, including all taxa, and it was evident that the populations graded into each other because it was not possible to differentiate between taxa as a result of the lack of unique alleles (Fig. 3b).

Nei's (1973) coefficient of gene differentiation relative to the total population (G_{ST}) is equivalent to Wright's (1978) fixation index (F_{ST}). These values averaged 0.643 and 0.638, respectively, for the *Sutherlandia* populations and taxa studied (excluding outgroups). This indicates that the F_{ST} values are similar at these taxonomic levels, i.e.,

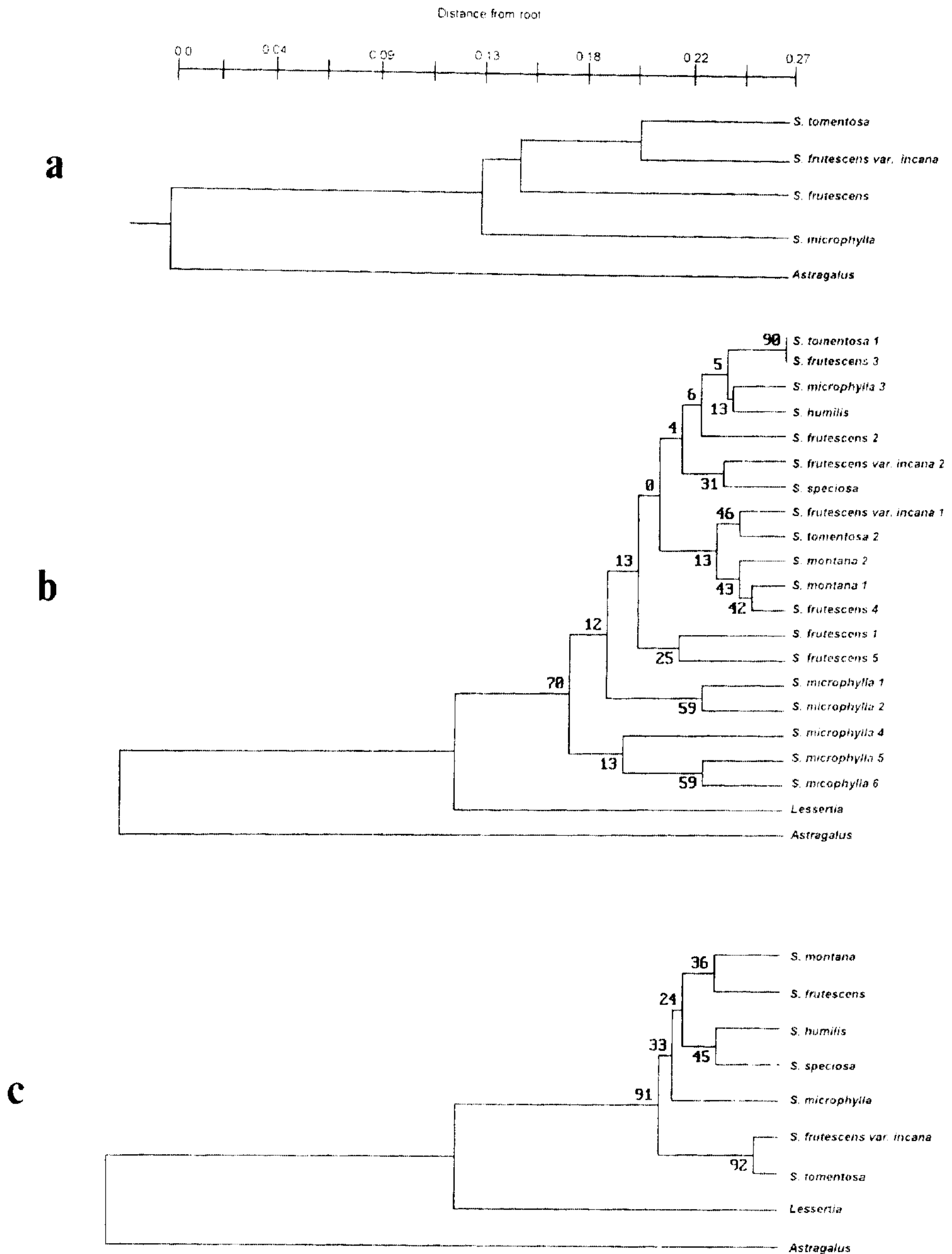

Fig. 3. Dendrograms based on Nei's (1978) unbiased genetic distance showing relationships of (a) seven populations (SF4, SF5, SV1, SM4, SM5, SM6, ST2) from four taxa of *Sutherlandia* and outgroup *A. atropilosulus* subsp. *burkeanus*, (b) nineteen populations from seven taxa and (c) seven taxa of *Sutherlandia* and two outgroups (*A. atropilosulus* subsp. *burkeanus* and a *Lessertia* species) based on allozyme data. Bootstrap numbers are presented at the nodes.

there is as much differentiation among subpopulations from the same taxon compared to subpopulations from different taxa. For example, the F_{ST} value is 0.767 for the SF populations and 0.597 for the SM populations. These F_{ST} values indicate a reduction in heterozygosity between subpopulations studied due to random genetic drift since values of F_{ST} are close to zero if all subpopulations are in Hardy–Weinberg equilibrium with the same allele frequencies (Nei, 1986). The F_{IT} values of 0.876, 0.920 and 0.877 for the above-mentioned populations, respectively, which quantifies inbreeding due to population subdivision, are also indicative of low levels of gene flow between the populations studied, but it may reflect adaptation to different ecological conditions.

Average D values (Table 4) between taxa (0.032) is lower than between populations (0.077), and genetic distances of the same magnitude between three taxa of *Sutherlandia* (*S. frutescens*, *S. microphylla* and *S. montana*) were obtained. Taxa differences were identified between ST1, SV1, SM4 and A at *GPI-1*a*, **b* and **c* (Fig. 2a). Despite the taxa differences observed (Fig. 2b) there are no distinct allozymes (or allele frequencies) or geographic patterns of allele frequencies between taxa at different populations to distinguish them.

The phylogenetic tree (Fig. 3b) illustrates the genetic relationships between *Sutherlandia* taxa; ST and SV formed a consistent group (92% bootstrap value). This grouping is sensible because SV is morphologically an intermediate between SF and ST. These two taxa co-occur and both have dense pubescent leaves. *Sutherlandia speciosa* (SS) is endemic to the Khamiesberg in Namaqualand and is grouped with *S. humilis* (SH) (Fig. 3c) from the eastern Karoo regions. Morphologically, *S. speciosa* is a spreading shrub of 0.4 m with large flowers and the stipe curving downwards in the mature pods. It is similar to *S. frutescens* (SF), except for the stated pod character and the larger flowers and habit. In contrast, *S. humilis* is a prostrate shrublet of less than 0.2 m tall and is also similar to *S. frutescens*, again except for the habit. It seems likely that *S. humilis* (SH) and *S. speciosa* (SS) are mere varieties of *S. frutescens* (SF), adapted to different geographical and environmental conditions (Fig. 3c). The same may be true of *S. speciosa* (SS) and *S. montana* (SMO), the only two "taxa" found at high altitudes, both with exceptionally large flowers. *Sutherlandia microphylla* (SM) is grouped separately from the other taxa on the basis of the oblong pods (length to width ratio of more than two) and narrow leaflets.

Liston (1992) studied *Astragalus* taxa and concluded that morphological and allozyme divergence patterns are concordant and that infraspecific taxa are characterised by slight morphological and little or no genetic differentiation. The taxa however, exhibited more pronounced morphological and allozyme differentiation as compared to conspecific populations. Although these observations were in agreement with the taxonomic treatment of Barneby (1964) and supported the validity of his taxa concepts in *A.* sect. *Leptocarpi* subsect. *Californici*, the results of the UPGMA phenogram suggested that taxonomic categories above taxa level do not reflect genetic relationships. Liston's (1992) findings are similar to the results shown in Fig. 3a, but not to those in Fig. 3b and c. It is clear that *Sutherlandia* populations show almost no agreement between morphology, geographic origin and isozyme patterns. A similar lack of agreement between morphology and isozyme patterns was also

found in other genera of the tribe Galegeae. Hornero and Pèrez (1997) were able to illustrate geographical relationships among 23 *Colutea* populations using cluster analysis, but a strong correlation was lacking between genetic and geographic distance within each area. Populations from the same area were clustered together and two major gene pools appeared among the Iberian *Colutea* populations studied, but these did not agree with the classification proposed on the basis of morphological criteria. The paper by Hornero and Pèrez (1997) seems particularly relevant for comparison since *Colutea* is the closest relative of *Sutherlandia* for which published information could be found. It is interesting to note the same lack of agreement between the current taxonomy of *Sutherlandia* and the enzyme patterns.

4. Conclusions

Our results indicate that the *Sutherlandia* taxa have very low genetic differentiation as compared to populations. This observation is consistent with the taxonomic treatments of Meyer (1836) and Harvey (1933) who regarded the South African *Sutherlandia* genus as one variable taxon, *S. frutescens*. On the other hand, Phillips and Dyer (1934) were inclined to the view that they were dealing with a large taxon complex which was separating into definite taxa in different geographical areas. In each of these areas they found that the fruits had differentiated sufficiently to justify their grouping at specific rank, but one would tend to argue against the validity of using only one characteristic in taxa delimitation.

Our survey of populations from all the taxa illustrated an integration of populations and taxa which cannot be distinguished from each other by allozyme data. The results of this study, therefore, suggest that a more conservative taxonomic classification system for the genus *Sutherlandia* is called for. However, ongoing morphological investigation can affect final decisions regarding the circumscription and rank of the various geographical forms of *Sutherlandia*. It is already clear that the specific rank is inappropriate in many cases, and that the rank of subtaxa or variety will give a better reflection of the lack of genetic differentiation and the lack of morphological discontinuities between some of the forms hitherto accepted as distinct taxa.

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