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Evolution of sprouting versus seeding in *Aspalathus linearis*

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Abstract. We have tested the hypothesis that reseeding is a plesiomorphic character state and that sprouting is a derived state in *Aspalathus linearis*, and that the latter is an adaptation to ensure fire-survival in a fireprone environment. Samples of five seeder and four sprouter populations of A. *linearis* were examined by horizontal starch gel electrophoresis to assess the amount of genetic differentiation within and between sprouting and seeding populations, and to determine the extent of gene flow between the populations. Leaf extracts were surveyed for ten enzymes and gene products revealed genetic variation at 13 (76%) of 17 protein coding loci. Allele frequency differences were found between sprouting and seeding populations and genetic distance values show that the sprouters are grouped separate from the seeders, thus providing support for the morphological data on which the above mentioned hypothesis is based. It is evident that evolution operates at the population level in *A. linearis*.

Key words: Fire-survival strategy, Crotalarieae, *Aspalathus linearis*, seeders, sprouters, electrophoresis.

Aspalathus linearis (Burm. f.) Dahlgr. (Rooibos Tea) is a commercially important Cape legume indigenous to the Fynbos region of South Africa, which is a fire-controlled vegetation where reproduction is largely driven by recurrent fires (Cowling 1987, Le Maitre and Midgley 1992). The two main fire-survival strategies in Cape legumes and other Fynbos plants were described by Schutte et al. (1995), namely sprouters and seeders. Sprouters are plants that are able to coppice from an underground lignotuber after fire, resulting in a multi-stemmed appearance at ground level. In contrast, seeders are killed by fire and can only recruit from the soil-borne seed bank after fire. They usually have a single main stem at ground level. Intervals between fires vary from four to 40 years, but fires usually occur at 12 to 20 year intervals. Regeneration of all species takes place within the first year after a fire. These fluctuations in fire frequency have influenced the evolution in Fynbos plants because only taxa that are adapted to fire survive (Linder 1985, Cowling 1987).

Aspalathus linearis is an exceptionally variable species and has been treated taxonomically in different ways (Dahlgren 1968, 1988). The species comprises a range of partially allopatric populations with distinct morphological differences. Only one study was previously done to document the genetic diversity and population structure of Rooibos Tea (Van der Bank et al. 1995a). Of particular interest is the fact that some populations are

Table 1. Morphological and biochemical characters and character states used for the cladistic analysis of eight populations of *A. linearis*, using *A. pendula* as outgroup. The partially resolved cladogram generated from the morphological data set is shown in Fig. 2. See Fig. 1 for localities

Population number:	Population and form:	Voucher:	1	2	3	4	5	6	7	8
Outgroup										
Aspalathus pendula		BVW 2998, RAU	0	0	0	0	0	0	0	0
Aspalathus linearis										
Seeders										
1	Pinifolia form, Wupperthal	BVW 3627, RAU	1	0	1	2	2	1	2	0
2	Pinifolia form, Biedouw	BVW 3628, RAU	1	0	1	1	2	1	2	0
3	Rocklands, Pakhuis Pass	BVW 3630, RAU	1	0	1	1	1	0	1	0
4	Kriedouw, Kriedouw	BVW 3624, RAU	1	0	1	1	1	0	1	0
5	Arborescent form, Duiwelskop	BVW 3622, RAU	0	0	0	1	1	0	1	0
Sprouters										
6	Decumbent form, Gifberg	BVW 3613, RAU	2	1	1	2	1	0	2	1
7	Grey form, Elandskloof	BVW 3631, RAU	1	1	1	1	1	0	1	1
8	Decumbent form, Franschhoek	BVW 2998, RAU	2	1	1	2	1	0	2	1

Characters

1. HABIT:
   Erect, single-stemmed, >2m high = 0
   Erect, multi-stemmed, 0.5–1.5m high = 1
   Decumbent, multi-stemmed = 2

2. FIRE-SURVIVAL:
   Reseeder = 0
   Resprouter = 1

3. LEAF MAXIMUM LENGTH:
   long (up to 80 mm) = 0
   medium (up to 40 mm) = 1
   short (up to 30 mm) = 2

4. FLOWER SIZE
   (expressed as carina length):
   large (7 to 8 mm) = 0
   medium (4 to 5 mm) = 1
   small (3 mm) = 2

5. FLOWER COLOUR:
   Bright yellow = 0
   Yellow and violet = 1
   Dark violet = 2

6. CARINA APEX:
   Normal = 0
   Apiculate = 1

7. POD MAXIMUM LENGTH:
   Long (up to 25 mm) = 0
   Medium (15 to 18 mm) = 1
   Short (9 to 13 mm) = 2

8. **PER-3** LOCUS:
   B-allele present = 0
   B-allele absent = 1

seeders, while others are sprouters. Field observations over many years have shown that the two fire-survival strategies are genetically fixed at the population level. The aim of this study was to explore the evolutionary history of seeding versus sprouting populations of *A. linearis* using allozyme electrophoresis.

Material and methods

Morphological analysis. A cladogram was generated by using the computer software package PAUP (Swofford 1985). A tree search was done using a heuristic search with 1000 random sequence additions and TBR (tree bisection-reconnection) branch swapping. Internal support was determined by bootstrapping (1000 replicates). *A. linearis* is one of four species of a very distinct infrageneric group (Dahlgren 1988) characterised by needle-like leaves. *A. pendula* and *A. lebeckioides* Dahlgr. are the two closest relatives of *A. linearis* and are distinguished from it only by minor morphological details. *A. lebeckioides* is a rare species endemic to the coastal plains at Bredasdorp and was not available for study. Character states were polarized using the method of outgroup comparison. The data set is shown in Table 1.

Genetic analysis. Leaf material of 216 individual plants collected from five seeder and three sprouter populations of *A. linearis* were analysed. The populations studied, voucher numbers and their geographical origin is depicted in Fig. 1. Collection, tissue preparation, extraction buffers, electrophoresis, staining of gels, interpretation of results, locus nomenclature and statistical analysis follow Van der Bank et al. (1995b). The following buffer systems were used: HC – a continuous buffer (pH = 6.5) system (Stuber et al. 1977); PO – a discontinuous buffer (electrode pH = 8.2; gel pH = 8.7) system (Poulik 1957); TC – a continuous Tris-Citric-Acid buffer (pH = 6.9) system (Whitt 1970); and TBE – a continuous Tris-Borate-EDTA buffer (pH = 8.6) system (Goncharenko et al. 1992).

Population genetic analyses were undertaken using the BIOSYS-1 computer package (Swofford and Selander 1989). The method used to determine relationships between the populations consisted of (a) interpreting electromorphs on gels in terms of Mendelian genetics, (b) computing allelic frequencies at various loci, and (c) converting allelic frequencies into a measure of genetic distance (D) among populations, using Nei's (1978) method. In addition to the latter calculations, levels of gene flow were also derived from Wright's (1978) F_{ST} values (measuring the total amount of differentiation among subpopulations relative to the limiting number of alleles under complete fixation). The F_{ST} values were subsequently used to estimate the effective number of genotypes exchanged between populations, N_M in each generation. The relationship between F_{ST} and N_M was determined using Wright's formula: $F_{ST} = 1/(1+4N_M)\alpha$, where $\alpha = (n/(n-1))^2$ and n is the number of populations analysed (Takahata 1983). This equation assumes that F_{ST}, for neutral alleles, is nearly independent of both the mutation rate and the number of populations (Crow and Aoki 1984).

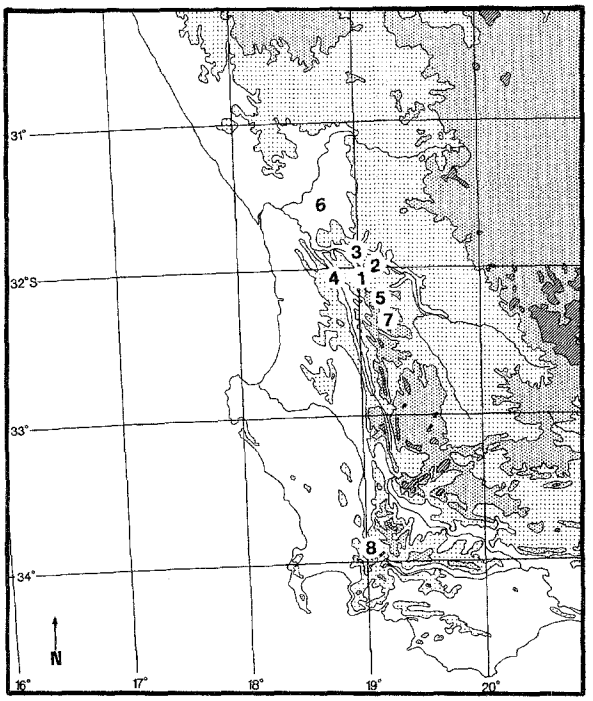

Fig. 1. The natural distribution of *A. linearis*, showing the localities of the populations studied. Seeding populations: (1) Wupperthal, (2) Biedouw, (3) Rocklands, (4) Kriedouw, (5) Duiwelskop; sprouting populations: (6) Gifberg, (7) Elandskloof, and (8) Franschhoek Pass

Results and discussion

Morphological data

Habit. There is considerable variation in the growth form of *A. linearis*, varying from prostrate or decumbent to ascending or erect, and from quite low to several meters high. All of the populations studied have shrub-like habits except for the arborescent form from Duiwelskop. All the seeder populations as well as the grey sprouting tea type (Citrusdal) are erect, single-stemmed at ground level but multi-stemmed higher up and between 0.5 and 1.5 m high, whereas the other two sprouter populations (Gifberg and Franschhoek) are decumbent and multi-stemmed at ground level.

Fire-survival. According to Schutte et al. (1995) the two fire-survival strategies, sprouting and seeding, are important taxonomic characters at the generic and specific level and very few species have both sprouter and seeder populations as in the case of *A. linearis*. Species exhibiting both strategies appear to have evolved independently in the different genera of the Podalyrieae and Liparieae (Fabaceae). In *A. linearis* however, the evolution of this character appears to take place at the population level. Observations over many years have shown that all populations can be classified as either seeding or sprouting and there are no intermediate populations known to us.

Leaves. The leaves are needle-shaped, 15 to 60 mm long and 0.4 to more than 1 mm thick. In some forms (e.g. Rocklands), the leaves turn rusty red and aromatic when dried.

Flowers. The flowers are solitary or up to 10 occur in racemes on branch tips. Petals are predominantly pale to bright yellow (Duiwelskop, Rocklands, Kriedouw, Elandskloof) or partly purple or violet (Wupperthal, Biedouw, Gifberg, Franschhoek Pass). The keel apex is obtuse and rarely has a tooth-like process at the tip (Wupperthal, Biedouw).

In the 1000 replicates of random taxon additions and subsequent swapping, two equally most parsimonious trees were identified (tree length = 14) with a consistency index (CI) of 0.86 and a retention index (RI) of 0.86. The strict consensus of these two trees is shown in Fig. 2, where bootstrap values are also shown. The fire-survival character (resprouting) and the absence of the B-allele at the PER-3 locus grouped the sprouting populations together. The Gifberg- and

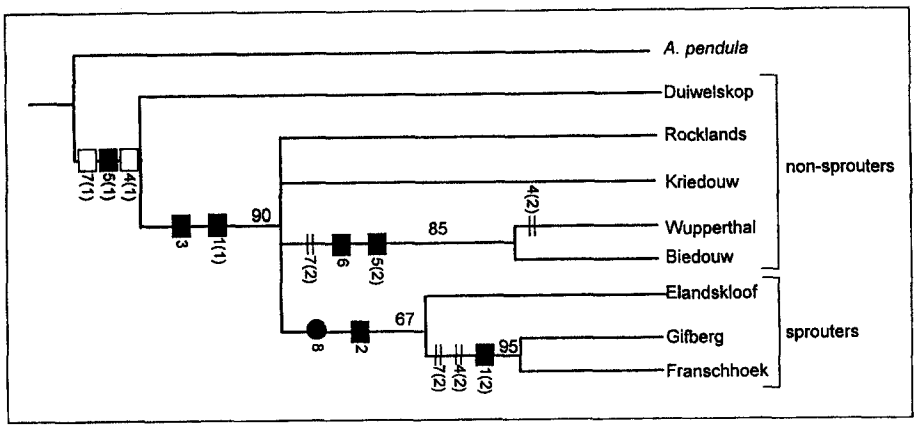

Fig. 2. A strict concensus tree showing the relationships between populations of *A. linearis* based on character states of morphological and allozyme data. See Table 1 for character states. ■ morphological data, ● allozyme data (character 8: loss of **B**-allele at the **PER-3** locus). Bootstrap values are given above each clade/node

Franschhoek Pass populations are sister to the Elandskloof population, which is interesting since these two populations are the only populations with a decumbent multi-stemmed habit.

Genetic data

Genetic interpretation of isozyme phenotypes were based on the quaternary structure of each enzyme and expression in other plant species. Seventeen enzyme coding loci provided interpretable results in all of the *A. linearis* populations analysed (Table 3). The data could be used for comparative studies, and to calculate the extent of differentiation between populations.

Genetic variation within populations. Genetic variation within populations was observed at 13 (76%) of the protein coding loci studied (Tables 2 and 3). Monomorphic loci are indicated in Table 2. The populations differed in the numbers and frequencies of alleles (Table 3). The Franschhoek population posesses unique alleles at the MNR-1 enzyme coding locus (A-allele) whereas all of the sprouting populations lack the B-allele at the PER-3 locus, and the dominant alleles differed for the sprouters (A-allele) compared to that of the seeders (B-allele) at the CAP locus. The distribution of total gene diversity within and among populations of *A. linearis* differs for the eight populations studied. For some populations, not all of the 13 polymorphic loci were variable thus the values of h at several loci in some populations is zero, and where polymorphism occurred, h values ranged from 0.044 to 0.598 (Table 3). These values compared favourably with results for four populations of *Virgilia oroboides* (Berg.) Salter subsp. *oroboides* (0.039–0.652) and four populations of *A. linearis* (0.039–0.595) reported by Van der Bank et al. (1995a, 1995b).

The maximum number of alleles for any given population was three, and the average heterozygosity (H) values ranged between 0.060 and 0.145 (Table 4). The mean H value for the seeder populations was 6.5% (the Rocklands populations had the lowest value of 4.0%) and much higher 11.97% for the sprouter populations (range: 9.0 to 14.5%). In a study done of genetic variation in a sprouter species, *Widdringtonia nodiflora* (L.) Powrie, versus two non-sprouter species, *W. cedarbergensis* Marsh and *W. schwarzii* (Marloth) Mast, Thomas and Bond (1997) found that the mean observed frequency of hetero-

Table 2. Enzymes, locus abbreviations, enzyme commission numbers (E.C. No.) and buffers used in the study of *A. linearis*.

Enzyme	Locus	E.C. No	Buffer (pH)
Aspartate aminotransferase	*(**AAT**)	2.6.1.1	**PO** (gel = 8.7; electrode = 8.2)
Acid phosphatase	*(**ACP**)	3.1.3.2	**HC**(6.5); **TC**(6.9)
Cytosol aminopeptidase	(**CAP**)	4.4.11.1	**TEB** (8.6)
Glucose-6-phosphate isomerase	(**GPI-1**;-2)	3.5.1.9	**TEB** (8.6); **PO** (gel = 8.7; electrode = 8.2)
Isocitrate dehydrogenase	(**IDH**)	1.1.1.42	**TC** (6.9)
Malate dehydrogenase	(**MDH-1**;-2;-3)	1.1.1.37	**HC** (6.5)
Menadione reductase	(**MNR-1**;-2)	1.6.99.-	**HC** (6.5)
Peroxidase	(**PER-1**;-2;-3)	1.11.1.7	**PO** (gel = 8.7 electrode = 8.2); **TEB** (8.6)
Phospho-glucomutase	(**PGM**)	5.4.2.2	**HC** (6.5)
Superoxide-dismutase	*(**SOD-1**;-2)	1.15.1.1	**TC** (6.9)

* = Monomorphic loci

Table 3. Relative allele frequencies (X_2) efficients of heterozygosity deficiency or excess (d) and individual heterozygosity (h) values at polymorphic loci for eight populations of *A. linearis*

		Populations							
		Seeders					Sprouters		
Locus	Allele	1	2	3	4	5	6	7	8
GPI-1	fA	0.045	0.091	0.045	0.159		0.057	0.033	0.150
	fB	0.955	0.909	0.955	0.841	1.000	0.943	0.967	0.850
X_2		22.000	22.000	22.000	15.160		53.000	30.000	20.000
d		−1.000	3.636	1.000	−0.830		−1.000	−1.000	−1.000
h		0.087	0.165	0.087	0.258		0.107	0.064	0.255
GPI-2	fA	1.000	1.000	1.000	1.000	1.000	0.625	1.000	0.500
	fB						0.375		0.500
X_2							1.440		0.333
d							0.600		−0.333
h							0.469		0.500
IDH	fA	0.971	1.000	1.000	1.000	1.000	0.750	1.000	1.000
	fB	0.029					0.250		
X_2		0.016					0.444		
d		0.030					0.333		
h		0.057					0.375		
CAP	fA					0.100	0.540	0.470	0.470
	fB	0.955	0.977	1.000	1.000	0.900	0.276	0.412	0.412
	fC	0.045	0.023				0.184	0.118	0.118
X_2		22.000	0.012			20.000	21.644	20.665	20.665
d		−1.000	0.977			−1.000	−0.351	−0.605	−0.605
h		0.087	0.044			0.180	0.598	0.595	0.595
MDH-1	fA	0.023				0.025		0.222	
	fB	0.977	1.000	1.000	1.000	0.975	1.000	0.778	1.000
X_2		0.012				0.013		0.735	
d		0.023				0.026		0.286	
h		0.044				0.049		0.346	
MDH-2	fA	0.023		0.068		0.050			
	fB	0.977	1.000	0.932	1.000	0.950	1.000	1.000	1.000
X_2		0.012		0.118		20.000			
d		0.023		−0.073		−1.000			
h		0.044		0.127		0.095			
MDH-3	fA	0.977	0.955	0.955	0.886	0.750	0.970	0.962	0.971
	fB	0.023	0.045	0.045	0.114	0.250	0.030	0.038	0.029
X_2		0.012	0.050	22.000	0.362	2.000	0.048	0.042	0.016
d		0.023	1.909	1.000	0.128	0.333	0.031	0.040	0.030
h		0.044	0.087	0.087	0.201	0.375	0.058	0.074	0.057
MNR-1	fA								0.167
	fB	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.833
X_2									0.120
d									0.200
h									0.278

Table 3 (continued)

		Populations							
		Seeders					Sprouters		
Locus	Allele	1	2	3	4	5	6	7	8
MNR-2	fA	0.038	0.346		0.269				0.500
	fB	0.962	0.654	1.000	0.731	1.000	1.000	1.000	0.500
X_2		0.021	0.294		2.223				0.333
d		−0.040	5.885		−0.414				−0.333
h		0.074	0.453		0.393				0.500
PGM	fA	0.545	0.841	0.786	0.841	1.000	0.900	0.778	0.833
	fB	0.455	0.159	0.214	0.159		0.100	0.222	0.167
X_2		0.153	5.290	1.802	0.499		0.062	0.735	0.120
d		0.083	5.886	0.293	−0.151		0.111	0.286	0.200
h		0.496	0.268	0.337	0.268		0.180	0.346	0.278
PER-1	fA		0.023			0.050			
	fB	1.000	0.977	1.000	1.000	0.950	1.000	1.000	1.000
X_2			0.012			0.055			
d			0.977			0.053			
h			0.044			0.095			
PER-2	fA	1.000	0.909	1.000	0.932	0.950	0.800	0.944	1.000
	fB		0.091		0.068	0.050	0.200	0.056	
X_2			0.220		0.118	20.000	5.000	0.031	
d			3.636		0.073	−1.000	1.000	0.059	
h			0.165		0.127	0.095	0.320	0.105	
PER-3	fA	0.955	0.905	0.976	0.864	0.975	1.000	1.000	1.000
	fB	0.045	0.095	0.024	0.136	0.025			
X_2		0.050	4.203	0.012	8.295	0.013			
d		−0.048	3.619	−0.024	−0.614	0.026			
h		0.087	0.172	0.046	0.236	0.049			

zygotes was lowest (2.5%) in *W. nodiflora* and higher in the seeder species (3.0% in *W. cedarbergensis* and 3.9% in *W. schwarzii*). Previous studies of flowering plants (dicotyledons) reported a mean *H* value of 5.9% (Nevo et al. 1984). The result of the present study compare favourably with these values except for the Rocklands population, where the value is much lower.

The percentage of polymorphic loci (*P*) range from 5.880 to 35.290 and the mean number of alleles per locus varied between 1.141 and 1.530 (Table 4). The seeder populations had an average value of $P = 22.35\%$. This value was higher for the sprouter populations (31.37%). Thomas and Bond (1997) reported a similar pattern, i.e. sprouters show a higher percentage of polymorphic loci than seeders. No obvious differences were found between the mean number of alleles per locus (*A*) in the sprouting compared to seeding populations (Table 4). In comparison with the other populations, little variation exists in the Rockland-, Wupperthal-, and Duiwelskop populations. The *P* values for the Wupperthal and Rocklands populations were 5.88% and 11.76% respectively, and for the other populations it was greater than 23.5%. Hamrick and Godt (1990) reported a *P* value of 20% for inbreeding species and an average *P* value of

Table 4. Mean number of alleles per locus (A), percentage of polymorphic loci (P) using the 0.95 criterion, average heterozygosity per locus (H), Nei's (1978) unbiased genetic distance values below diagonal between eight Rooibos Tea populations, F_{ST} values (bold) and N_m (italics) above the diagonal between seeders (populations 1 to 5) and sprouters (populations 6,7 and 8).

	Seeders				Sprouters			
Population	1	2	3	4	5	6	7	8
A	1.530	1.470	1.290	1.350	1.141	1.147	1.140	1.470
	±0.12	±0.12	±0.11	±0.12	±0.15	±0.15	±0.15	±0.12
P	5.880	29.410	11.760	35.290	29.410	35.290	23.530	35.290
H	0.060	0.082	0.040	0.088	0.055	0.124	0.090	0.145
	±0.029	±0.031	±0.021	±0.032	±0.024	±0.047	±0.042	±0.051
1	–	**0.076**	**0.037**	**0.066**	**0.137**			
		0.759	*1.627*	*0.884*	*0.393*			
2			**0.065**	**0.006**	**0.095**			
	0.010	–	*0.899*	*10.354*	*0.595*			
3				**0.050**	**0.080**			
	0.003	0.007	–	*1.1875*	*0.719*			
4					**0.073**			
	0.009	0.000	0.005	–	*0.794*			
5	0.016	0.011	0.005	0.009	–			
6							**0.065**	**0.111**
	0.042	0.043	0.037	0.043	0.032	–	*0.857*	*0.501*
7								**0.130**
	0.020	0.027	0.019	0.026	0.018	0.012	–	*0.418*
8	0.044	0.027	0.042	0.031	0.042	0.011	0.026	–

31.1% was reported by Nevo et al. (1984) for flowering plants. The results of the present study compare favourably with these averages exept for the Rocklands population. The *P* value for the Wupperthal population is also at least five times lower than values reported in the literature. 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 may account for the lower allozyme variation in the populations studied.

Short lived herbaceous species with a predominantly sexual, animal-outcrossing mode of reproduction and gravity dispersed seeds have a mean *H* value of 0.127, mean *P* value of 33% and an *A* value of 1.16 at the population level (Godt and Hamrick 1991). Thus, the values obtained for most of these parameters in this study are lower, with the Rocklands population having the lowest values (Table 4). However, the values for the Franschhoek population compare favourably with values listed in the literature. For example, long-lived species tend to maintain higher levels of genetic variation within their populations (Hamrick 1979), presumably because of the larger number of generations present in any given population (Levin 1978) and localised species also tend to have less variation than widely distributed species. This could explain the relatively high *H* values (9.0–14.5%) for the Gifberg, Elandskloof and Franschhoek populations (which are resprouters, and comprise various generations), compared to that of the seeding populations (4.0–8.8%).

Gene flow. The movement of genes between populations or population subdivisions has a significant influence on the

distribution of genetic variation. Species with restricted gene movement should exhibit greater infraspecific genetic differentiation than species with widely dispersed pollen and seeds. The available evidence, based primarily on the distribution of allozyme loci, supports this prediction (Brown 1979, Loveless and Hamrick 1984). There are, however, relatively few direct measures of gene flow in natural plant populations. Most attempts to measure gene flow involve following the movement of pollen and seed dispersal vectors or the actual movement of pollen or seed. The availability of methods to study allozyme loci has greatly improved estimates of gene flow. Allozyme loci have been used to: (1) follow the dispersal of a unique marker allele, (2) obtain estimates of gene flow from the distribution of allozyme variation among populations, and (3) identify maternal and paternal individuals using paternity analysis techniques. Many of the problems of estimating gene flow do not arise if the movement of unique alleles is followed directly. Since it is often difficult to find individuals with unique alleles, the procedure cannot be applied to all species or populations. In this study, the Franschhoek population possesses unique alleles at the MNR-1 locus whereas all of the sprouting populations lack the B-allele at the PER-3 locus. This loss of the B-allele at the PER-3 locus in all of the sprouting populations is a very important result since it is indicative that the sprouters are derived from the seeders (i.e. a part of the total gene pool was not transferred).

Schutte et al. (1995) speculated that there is a substantial difference in gene flow between some sprouting and seeding species of the Cape Fynbos legumes. Over time, gene flow between sprouting parents and their offspring may occur, since the parents are not killed by fire. On the other hand, temporal isolation in gene flow may occur in seeding taxa, as each new generation will be a cohort of its own. Thus, gene flow would be higher between sprouting generations compared to seeding generations. Our results (higher average P and H values, Table 4) support the above-mentioned hypothesis. It is, therefore, inferred that speciation would more readily occur in non-sprouters, as there can be no interbreeding between parents and seedlings after fire. In sprouters there would be a greater possibility for clinal variation. In the genus *Cyclopia*, for example, morphological differences between closely related seeding species, e.g. *C. plicata* Kies and *C. pubescence* Eckl. and Zeyh., are more marked than those between closely related sprouting species (e.g. *C. bolusii* Hofmeyer and E. Phillips and *C. aurescens* Kies) (Schutte et al. 1995). This could be ascribed to a more rapid rate of speciation and differentiation within seeders compared to sprouters. Individuals of most seeding species are relatively short-lived. In long-lived species gene flow may occur between successive generations, since interfire periods may not be long enough to lead to the isolation of populations. According to Schutte et al. (1995), one would expect a higher number of seeding than sprouting taxa within different genera. Their data did not support the concept, since within habitat specialists there is usually an equal number of sprouting and seeding species. They proposed that the extinction rate of populations within seeding taxa is much higher than in sprouting taxa, which reduces the expected number of seeding habitat specific taxa.

There are a few indirect methods of estimating gene flow, but all of these methods depend on the distribution of allele frequencies among populations (Wright 1951; Slatkin 1981, 1985; Barton and Slatkin 1986). Isozyme loci are the traits of choice for studies of gene flow since they are unlikely to be affected by pollinator activity or by the behavior of seed dispersal agents, are generally available for all species and can be easily scored. Estimates of gene flow generally reflect the patterns revealed by hierarchical fixation (F) index statistics. An estimate of $N_m = 1$ indicates that one migrant arrives every generation

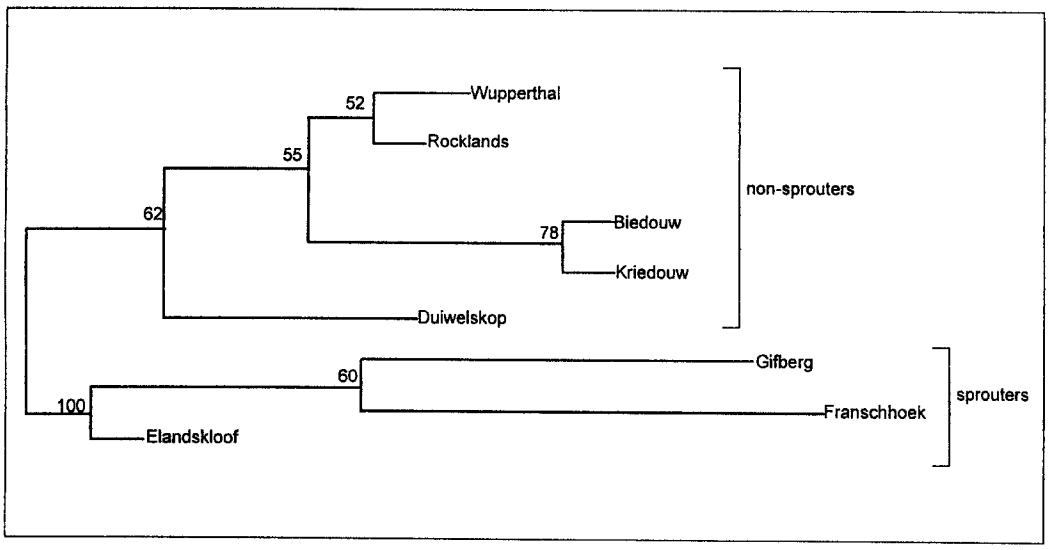

Fig. 3. Dendrogram of relationships between populations of *A. linearis* using neighbor-joining and bootstrapping, and Nei et al's (1983) genetic distance values. Bootstrap values are given above each node

to a population, and $N_m < 0.5$ suggests that gene flow is too low to prevent genetic divergence among populations due to genetic drift (Wright 1951). For example, mean estimates of N_m calculated by Hamrick and Griswold (1989) ranged from 0.065 for selfing species to 5.38 for outcrossed wind-pollinated species. Mixed-mating and outcrossed animal-pollinated species had intermediate N_m values. In the present study relatively low levels of gene flow was found between the populations (Table 4) except for the Biedouw and Kriedouw populations were it was very high ($N_m = 10.354$). Gene flow among the Elandskloof-and Franschhoek Pass populations ($N_m = 0.418$), and Wupperthal and the Duiwelskop ($N_m = 0.393$), appears to be too low to counteract the effect of genetic drift. However, differences between samples may merely indicate allele frequency fluctuations (Berg and Gall 1988), and low F_{ST} values for neutral loci do not necessarily imply the absence of genetic divergence at other loci subjected to natural selection (Chakraborty and Leimar 1987). This may explain why no obvious differences in gene flow could be found between the seeding and sprouting populations (Table 4).

Cluster analysis of genetic distance using UPGMA. The D values (Table 4) ranged from zero to 0.044 between populations, with the smallest D value between the Biedouw and Kriedouw populations and the largest value calculated between Wupperthal and Franschhoek Pass respectively. The D values averaged 0.008 between seeders and 0.016 between sprouters, and 0.033 between seeders and sprouters. These differences are reflected in the dendrogram (Fig. 3), which shows that the sprouters were grouped separate from the seeders.

Conclusions

Congruence between different data sets is desired in order to resolve phylogenetic relationships. The cladogram represented in Fig. 2 is a consensus tree, showing two characters present in seeder populations and the outgroup which are lost in sprouting populations: the absence of the B-allele at the PER-3 locus and the fire-survival strategy (sprouting). This implies that seeders are ancestral and that sprouting has developed from seeding as a fire-survival strategy. The cladogram shows that the change to sprouting

occurred only once in *A. linearis*, but more sprouting populations should be studied to confirm this result. In other cladistic studies of Cape legumes, sprouting was usually not congruent with other characters, suggesting that a switch to sprouting and back to seeding is possible, at least in *Cyclopia* Vent., *Podalyria* Willd. and *Hypocalyptus* Thunb. (Schutte 1995, Schutte et al. 1995). The pattern of diversity amongst *A. linearis* populations suggests abrupt genetic changes, perhaps as a result of strong selection and/or genetic bottlenecks. Whether the change from seeding to sprouting was really a single evolutionary event or whether it was convergent in different populations remains to be demonstrated convincingly.

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