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Genetic Variation Within and Geographical Relationships Between Four Natural Populations of *Virgilia oroboides* (Tribe Podalyrieae: Fabaceae)

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Key Word Index—Fabaceae; Cape legumes; *Virgilia oroboides*; genetic diversity; protein electrophoresis; geographical variation; genetic distance.

Abstract—*Virgilia oroboides* has a large indigenous range encompassing most of the south-western coastal areas of the Cape Province, South Africa, where it exhibits considerable morphological variation. Four populations of *V. oroboides* were examined by horizontal starch gel electrophoresis to assess levels and patterns of genetic variation and to estimate the amount of genetic differentiation among populations at 37 protein coding loci. Leaf extracts were surveyed for 22 proteins, and gene produts of 37 loci revealed genetic variation at 17 (46%) thereof. The percentage of polymorphic loci range from 29.73–43.24% (0.95 criterion), values of 1.35–1.49 ($\pm$ 0.09) were obtained for the mean number of alleles per locus, and average heterozygosities per locus were calculated at 0.127–0.198. No statistical significant difference between heterozygosity values was obtained when fewer (25 compared to 50) individuals were analysed. The mean genotypic distance index (Nei, 1978 = 0.0185) suggests a low degree of differentiation between populations. Geographic relationships reflect gradual isolation of the western populations, which may have become restricted to a few moist sites as a result of a decrease in total forest area in response to long-term climatic changes. This isolation and subsequent selection may have resulted in a depletion of genetic variation from the eastern to the western regions. Estimates of elapsed divergence times suggest that these populations diverged 0.02–0.18 million years ago. Copyright © 1996 Elsevier Science Ltd

Introduction

Virgilia (Fabaceae: Podalyrieae) is a genus of small trees endemic to the south-western and southern coastal areas of South Africa. As forest margin relicts, their discontinuous natural distribution pattern is closely associated with the distribution of afromontane forest, and the populations are mostly limited to moist sites on the ecotone between forest and fynbos.

Three closely related taxa (two species and two subspecies: *V. oroboides* (Berg.) Salter ssp. *oroboides*, *V. oroboides* ssp. *ferruginea* B.-E. Van Wyk and *V. divaricata* Adamson) were recognised (Van Wyk, 1986), based mainly on the texture of the bark, the pubescence and shape of the leaves, the size and persistence of bracts, the colour of the flowers (notably the presence or absence of a distinct pollen guide on the keel petal), the size of the seeds and the time of flowering (spring *vs* summer). *Virgilia oroboides* ssp. *oroboides* occurs along the southern slopes of the Langeberg (Swellendam area) and westwards to the Cape Peninsula. The eastern populations often comprise several hundred individuals, with only small distances between populations. Towards the west, however, populations are more isolated from one another and their sizes decrease dramatically. The reproductive biology of *Virgilia* is poorly known, but circumstantial evidence (morphological variation, pollination mechanism and biogeographic patterns) suggests recent allopatric speciation in *Virgilia*.

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Enzyme electrophoresis was used to describe and quantify genetic diversity within *V. oroboides* (Van der Bank *et al.*, 1995). These authors proposed a wider survey of all the taxa (including samples from various geographical regions and morphologically distinct populations). The purpose of the present study is to describe genetic variation and geographical relationships of four different *V. oroboides* ssp. *oroboides* populations, to infer colonisation patterns and to determine the extent of genetic differentiation between populations. Another objective was to estimate the time of divergence using molecular models and biogeographic data.

Materials and Methods

Plant material. Leaves from actively growing shoots were collected from four populations of *V. oroboides* ssp. *oroboides* (Fig. 1), all sampled in the early spring of 1994. Fifty trees were sampled from a natural population at Tradouws Pass (33°57'S, 20°42'E; altitude: 340 m). This population was chosen because it is geographically isolated from other taxa of *Virgilia* (both natural and cultivated) so that there is no possibility of hybridisation or introgression. Furthermore, this population is morphologically uniform, suggesting low levels of outcrossing with other populations.

The population size sampled from Swellendam (34°00'S, 20°27'E; altitude: 300 m) comprised 25 individuals, and 31 and 25 individuals were sampled respectively from Betty's Bay (34°21'S, 18°55'E; altitude: 140 m) and Kirstenbosch (33°58'S, 18°26'E; altitude: 200 m). The approximate distances between these populations are: Tradouws Pass to Swellendam *ca* 12 km, Swellendam to Betty's Bay *ca* 150 km and Betty's Bay to Kirstenbosch *ca* 65 km. The Tradouws Pass and Swellendam populations are morphologically similar in that the trees are large with spreading crowns; they have a coarse and fissured

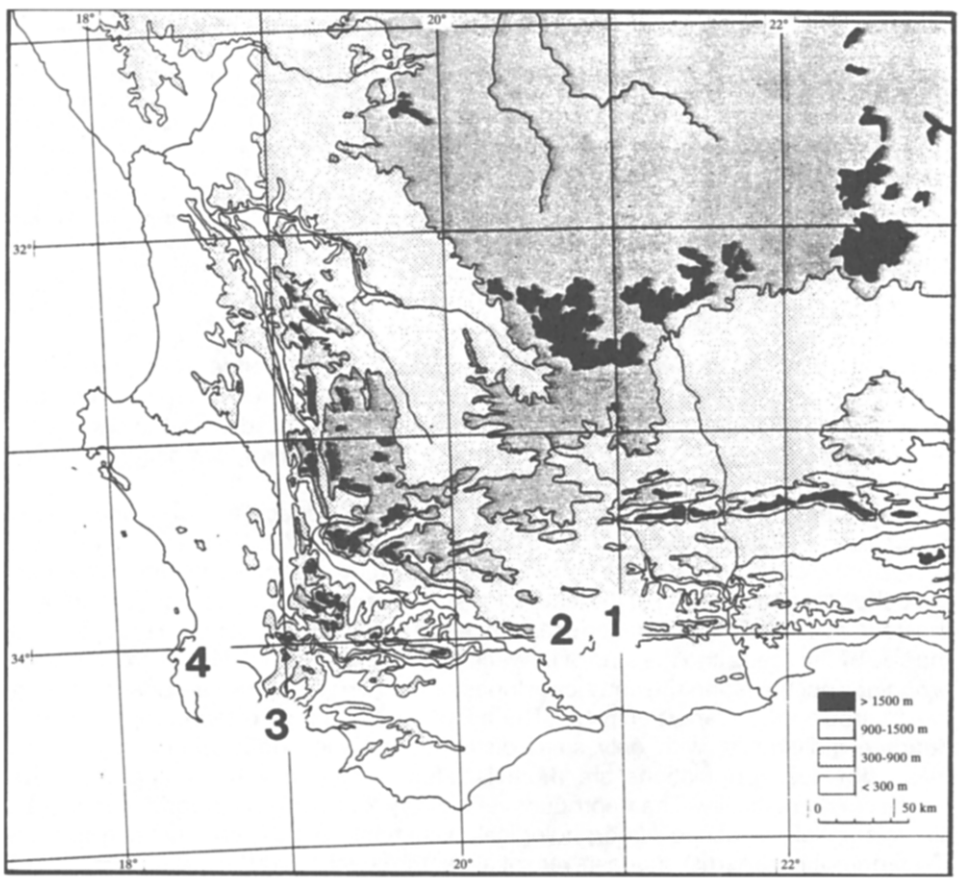

FIG. 1. POPULATIONS OF *V. OROBOIDES* SSP. *OROBOIDES* SAMPLED: 1 = TRADOUWS PASS; 2 = SWELLENDAM; 3 = BETTY'S BAY; 4 = KIRSTENBOSCH.

bark, white sericeous leaflets, exceptionally large floral bracts and pale pink flowers. However, the bracts are larger and the leaflets are more hairy in the Tradouws Pass population. Whereas the Tradouws Pass population comprises several hundred individuals, the Swellendam, Betty's Bay and Kirstenbosch population sizes are much smaller, with only about fifty individuals in each. At all these sites, the trees are restricted to a small area along a stream. The trees at Betty's Bay are relatively tall and straight (up to 8 m high), with a variable bark (some coarse, some smooth), less densely hairy leaflets than at Swellendam and the flowers pale pink or white. At Kirstenbosch, the trees are small (3–6 m high), with relatively smooth bark; the bracts are rather small and somewhat caducous, and the flowers are pink, some with the base of the standard petal marked with dark pink.

Procedure. Collection, tissue preparation, extraction buffers, electrophoresis, staining of gels, interpretation of results, locus nomenclature and statistical analysis follow Van der Bank *et al.* (1995). The supporting medium for horizontal starch gel electrophoresis consisted of 12% Sigma (S-4501) hydrolysed potato starch. We used the following buffer systems: **CB**: a discontinuous buffer (electrode pH = 8.1; gel pH = 8.4) system (Cooke and Buckley, 1987); **CT**: a continuous buffer (pH = 6.1) system (Clayton and Tretiak, 1972); **MF**: a continuous buffer (pH = 8.6) system (Markert and Faulhaber, 1965); **P**: a discontinuous buffer (electrode pH = 8.2; gel pH = 8.7) system (Poulik, 1957); **RW**: a discontinuous buffer (electrode pH = 8.0; gel pH = 8.7) system (Ridgway *et al.*, 1970); and **TC**: a continuous tris, citric acid buffer (pH = 6.9) system (Whitt, 1970). We stained for 22 proteins (Table 1).

By increasing the width of gels from 13 to 14 cm (to allow for better migration of allelic products), we were able to identify three alleles at the *CAP-2* protein coding locus. We compared average heterozygosity ($\bar{H}$) values obtained for the largest population (Tradouws Pass) with values obtained for the first 25 individuals analysed from the same population. Archie's (1985) statistical test for independent sample comparisons of heterozygosity data was used to determine if small sample sizes affect estimates of $\bar{H}$ drastically in *V. oroboides*.

We used *CONSENSE* (Felsenstein, 1993) to construct a dendrogram from Wagner trees using *PAUP* and binary coded allele data (Swofford, 1985), *FREQPARS* (Swofford and Berlocher, 1987) to infer a parsimony tree directly from allelic frequencies, and *UPGMA* (Sneath and Sokal, 1973) to produce phenograms using Nei's (1978) and Cavalli-Sforza and Edwards' (1967) genetic distances.

Divergence times were estimated from allozyme data by using Nei's (1987) formula, where time = genetic distance/2 (the substitution rate per locus per year). If the latter value is set at 10^{-7}, then time may be calculated as $5 \times 10^6 \times$ genetic distance.

TABLE 1. LOCUS ABBREVIATIONS AND ENZYME CODE NUMBERS (E.C. No.) ARE LISTED AFTER EACH PROTEIN. See Materials and Methods for abbreviations of buffers used

Protein	Locus	E.C. No.	Buffer
Acid phosphatase	*ACP-1, -2*	3.1.3.2	CT
Adenylate kinase	**AK*	2.7.4.3	TC
Aspartate aminotransferase	*AAT*	2.6.1.1	MF
Cytosol aminopeptidase	**CAP-1, -4*	3.4.11.1	CB, CT
	CAP-2		MF
Esterase	**EST-1, -2, 5, -6, -8*	3.1.1-	RW, P
	EST-3, -4, 7, -9		
General protein	**PROT-1, -2*		MF
Glucose-6-phosphate isomerase	*GPI*	5.3.1.9	RW, P
Guanine deaminase	**GDA*	3.5.4.3	CT
Isocitrate dehydrogenase	**IDH*	1.1.1.42	TC
Malate dehydrogenase	**MDH-1, -2*	1.1.1.37	TC
Malic enzyme	**ME*	1.1.1.38	RW
Menadione reductase	**MNR*	1.6.99.2	MF
Peptidase:		3.4.-	
Substrate:			
Glycyl-L-leucine	**PEP-A*		RW
Leucylglycylglycine	*PEP-B*		MF
Leucine-alanyl	*PEP-C*		MF
L-Phenylalanyl-L-proline	**PEP-D1, -D2*		RW
Leucyl-tyrosine	*PEP-S*		MF
Peroxidase	*PER-1, -2*	1.11.1.7	MF, P
6-Phosphogluconate dehydrogenase	*PGD*	1.1.1.44	RW
Purine-nucleoside phosphorylase	**NP*	2.4.2.1	CB
Shikimate dehydrogenase	*SKDH*	1.1.1.25	MF
Superoxide dismutase	*SOD*	1.15.1.1	RW

* = Monomorphic loci.

Results

Thirty-seven protein coding loci provided interpretable results in all *V. oroboides* populations analysed, and these data could be used for comparative studies and to calculate the extent of differentiation between populations. Twenty of the 37 loci (54%) displayed monomorphic gel banding patterns (Table 1). Allozyme frequencies for loci where significant ($P < 0.05$) deviations of alleles from expected Hardy–Weinberg proportions occurred and individual heterozygosities (h) are listed in Table 2, which lists populations geographically from east (Tradouws Pass) to west (Kirstenbosch). Allelic frequencies did not deviate from Hardy–Weinberg expectations (Table 2) at any of the loci, simultaneously for all of the *V. oroboides* populations studied. However, relatively large deficiencies of heterozygotes and deviations of allele frequencies from expected Hardy–Weinberg proportions were encountered at the *ACP, CAP-2, EST-7, -9, GPI, PEP-B, -C, PER-2* and *SOD* protein coding loci (Table 2), especially for the population from Tradouws Pass. Individual heterozygosity values ranged from 0.039 for *GPI* to 0.652 for *CAP-2*, both in Swellendam (Table 2).

The mean number of alleles per locus (A) was progressively reduced from the eastern population sampled (Tradouws Pass) towards the western regions (Table 3). This trend is also evident from the other population variation parameters (P and $\bar{H}$, respectively), except in the case of the Kirstenbosch population. The H value (Table 3) of the Tradouws Pass population ($N = 50$; $\bar{H} = 0.198$) did not differ significantly ($P < 0.05$) from the value obtained for the first 25 individuals ($\bar{H} = 0.177$) from the same population ($t = 0.123$; degrees of freedom = 72).

Genetic distance values ranged from 0.004 to 0.036 (Nei, 1978) and from 0.069 to 0.158 (Cavalli-Sforza and Edwards, 1967) between populations (Table 3), reflecting the same differentiation pattern obtained when a majority-rule consensus tree (Fig. 2) was constructed from trees produced using *FREQPARS, PAUP* and *UPGMA* as outlined in the Materials and Methods section. The populations from Kirstenbosch and Betty's Bay were grouped together (75% majority), and these populations were separated from the Swellendam and Tradouws Pass populations respectively (100% majority in both of the latter instances). Divergence times ranged from 0.02 to 0.18 (average = 0.0925) million years.

Discussion

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). Deviations of allele frequencies from expected Hardy–Weinberg proportions occurred at the *ACP, CAP-2, EST-7, -9, GPI, PEP-B,* -C, *PER-2* and *SOD* loci (Table 2) in the Tradouws Pass population. Deficits of heterozygotes were obtained at these loci (Table 2), indicating the occurrence of scarce alleles, as discussed by Van der Bank *et al.* (1995). Hardy–Weinberg proportions of allele frequencies were, however, obtained at the *AAT, EST-3, -4, PEP-S, PER-1, PGD* and *SKDH* protein coding loci. Less deviations of allele frequencies from expected Hardy–Weinberg proportions were obtained at loci in the other populations (Table 2) compared to those of the Tradouws Pass population, indicating that these populations were colonised by founders with limited genetic variation or that selective pressures in the smaller populations resulted in a gradual decrease in genetic variation.

No statistically significant difference between heterozygosity values was obtained when fewer individuals were analysed. A similar result was obtained by Nei and Roychoudhury (1974) from a statistical and theoretical study of man, based on

TABLE 2. RELATIVE ALLELE FREQUENCIES, χ^2-VALUES, COEFFICIENTS OF HETEROZYGOSITY DEFICIENT OR EXCESS (d) AND INDIVIDUAL HETEROZYGOSITY (h) VALUES FOR EACH POLYMORPHIC LOCUS. FOR EACH COLUMN, THE NUMBER OF INDIVIDUALS STUDIED IS GIVEN (WITH VALUES IN BRACKETS FOR THE FIRST 25 INDIVIDUALS FROM TRADOUWS PASS)

		Population			
Locus	Allele frequency	Tradouws Pass 50 (25)	Swellendam 25	Betty's Bay 31	Kirstenbosch 25
ACP-1	fA	0.375 (0.342)	0.219	0.260	0.526
	fB	0.625 (0.658)	0.781	0.740	0.474
χ^2		7.840* (3.278)	10.684*	5.766*	0.460
d		−0.467 (−0.415)	−0.817	−0.480	−0.156
h		0.469 (0.450)	0.342	0.385	0.499
ACP-2	fA	0.180		0.033	
	fB	0.820 (1.000)	1.000	0.967	1.000
χ^2		26.572*		0.036	
d		−0.729		0.034	
h		0.295		0.064	
AAT	fA	0.643 (0.688)	0.976	0.769	0.674
	fB	0.357 (0.312)	0.024	0.231	0.326
χ^2		2.928 (0.389)	0.012	3.185	0.277
d		−0.244 (−0.127)	0.024	−0.350	−0.110
h		0.459 (0.430)	0.046	0.355	0.440
CAP-2	fA	0.448 (0.417)	0.395	0.250	0.217
	fB	0.188 (0.167)	0.368	0.589	0.543
	fC	0.365 (0.417)	0.237	0.161	0.239
χ^2		12.262* (5.520)	6.736	15.515*	8.461*
d		−0.307 (−0.267)	−0.193	−0.114	−0.276
h		0.631 (0.625)	0.652	0.564	0.600
EST-3	fA	0.559 (0.455)	0.647	0.632	0.588
	fB	0.441 (0.545)	0.353	0.368	0.412
χ^2		0.925 (0.782)	0.878	0.172	0.781
d		−0.165 (−0.267)	−0.227	−0.095	0.214
h		0.493 (0.493)	0.457	0.465	0.484
EST-4	fA	0.292	0.400	0.500	0.278
	fB	0.708 (1.000)	0.600	0.500	0.722
χ^2		1.872	0.625	0.692	0.516
d		−0.395	0.250	−0.231	−0.169
h		0.413	0.480	0.500	0.401
EST-7	fA	0.952 (0.950)	1.000	1.000	1.000
	fB	0.048 (0.050)			
χ^2		9.476* (0.055)			
d		−0.475 (0.053)			
h		0.091 (0.095)			
EST-9	fA	0.629 (0.545)	0.800	1.000	1.000
	fB	0.371 (0.455)	0.200		
χ^2		9.121* (4.412*)	0.417		
d		−0.510 (−0.633)	−0.167		
h		0.467 (0.496)	0.320		
GPI	fA	0.770 (0.620)	0.980	0.953	1.000
	fB	0.230 (0.380)	0.020	0.047	
χ^2		7.178* (4.116*)	0.010	0.077	
d		−0.379 (−0.406)	0.020	0.049	
h		0.365 (0.471)	0.039	0.089	
PEP-B	fA	0.490 (0.542)	0.818	0.880	0.826
	fB	0.510 (0.458)	0.182	0.120	0.174
χ^2		5.111* (2.593)	0.543	9.648*	3.584
d		−0.320 (−0.329)	0.222	−0.621	−0.395
h		0.500 (0.497)	0.298	0.211	0.287

TABLE 2.—*CONTINUED*

Locus	Allele frequency	Population: Tradouws Pass 50 (25)	Swellendam 25	Betty's Bay 31	Kirstenbosch 25
PEP-C	fA	0.341 (0.441)	0.605	0.667	0.542
	fB	0.659 (0.559)	0.395	0.333	0.458
χ^2		8.588* (2.768)	0.851	0.429	2.593
d		−0.458 (−0.404)	0.212	−0.143	−0.329
h		−0.450 (0.493)	0.478	0.444	0.497
PEP-S	fA	0.436 (0.417)	0.750	0.643	0.844
	fB	0.564 (0.583)	0.250	0.357	0.156
χ^2		0.312 (0.960)	0.032	0.093	1.335
d		0.081 (0.200)	−0.048	−0.067	−0.289
h		0.492 (0.486)	0.375	0.459	0.262
PER-1	fA	0.551 (0.583)	0.761	0.827	0.886
	fB	0.449 (0.417)	0.239	0.173	0.114
χ^2		0.420 (0.020)	3.728	1.139	2.296
d		−0.093 (0.029)	−0.403	0.209	−0.323
h		0.495 (0.486)	0.364	0.286	0.201
PER-2	fA	0.340 (0.500)	0.271	0.529	0.444
	fB	0.660 (0.500)	0.729	0.471	0.556
χ^2		5.328* (1.636)	5.359*	1.446	1.901
d		0.339 (−0.273)	−0.473	−0.292	−0.325
h		0.449 (0.500)	0.395	0.498	0.494
PGD	fA	0.474 (0.700)	1.000	1.000	1.000
	fB	0.526 (0.300)			
χ^2		2.554 (1.372)			
d		−0.367 (−0.524)			
h		0.499 (0.420)			
SKDH	fA	0.402 (0.432)	0.380	0.750	0.531
	fB	0.598 (0.568)	0.620	0.250	0.469
χ^2		1.135 (0.917)	1.868	0.089	0.236
d		0.166 (0.204)	0.273	0.067	−0.122
h		0.481 (0.491)	0.471	0.375	0.498
SOD	fA	0.833 (0.938)	0.833	1.000	0.940
	fB	0.167 (0.062)	0.167		0.060
χ^2		8.112* (16.000*)	0.720		10.413*
d		−0.520 (−1.000)	−2.000		−0.645
h		0.278 (0.117)	−0.278		0.113

* = Loci where significant ($P<0.05$) deviations of alleles from expected Hardy–Weinberg proportions occurred.

TABLE 3. MEAN NUMBER OF ALLELES PER LOCUS (*A*), PERCENTAGE OF LOCI POLYMORPHIC (*P*) USING THE 0.95 CRITERION, AVERAGE HETEROZYGOSITY PER LOCUS (*H*), NEI'S (1978) GENETIC DISTANCE BELOW DIAGONAL AND CAVALLI-SFORZA AND EDWARDS' (1967) CHORD DISTANCE (ABOVE DIAGONAL) BETWEEN POPULATIONS

Population	Tradouws Pass		Swellendam	Betty's Bay	Kirstenbosch
	Total	First 25 individuals			
A	1.49 (± 0.09)	1.43 (± 0.09)	1.41 (± 0.09)	1.38 (± 0.09)	1.35 (± 0.09)
P	43.24	40.54	32.43	29.73	32.43
H	0.198 (± 0.038)	0.177 (± 0.039)	0.135 (± 0.033)	0.127 (± 0.032)	0.129 (± 0.034)
Tradouws Pass	—		0.137	0.158	0.158
Swellendam	0.024		—	0.097	0.090
Betty's Bay	0.036		0.008	—	0.069
Kirstenbosch	0.031		0.007	0.004	—

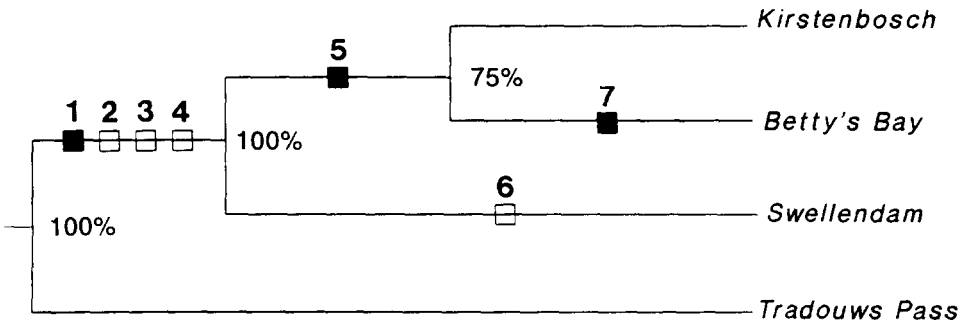

FIG. 2. MAJORITY-RULE CONSENSUS TREE PRODUCED FROM *FREQPARS*, *PAUP* AND *UPGMA* ANALYSES OF ALLOZYME DATA WITH LOSS OF ALLELES INDICATED. [■ = Common alleles; □ = scarce alleles (< 5%); 1 = *PGD*; 2 = *GPI*; 3 = *ACP-2*; 4 = *EST-7*; 5 = *EST-9*; 6 = *AAT*; 7 = *SOD*.]

empirical data. The latter authors determined that estimates of genetic variation are affected more by the number of loci studied than by the number of individuals. We are, therefore, confident that the number of loci reported in the present study (37) was sufficient to obtain an unbiased estimate of genetic variation from the relatively small numbers of individuals analysed in the Swellendam, Betty's Bay and Kirstenbosch populations ($N = 25$, 31 and 25, respectively).

Several factors may account for the lower allozyme variation obtained in the endemic populations from Swellendam, Betty's Bay and Kirstenbosch: 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. 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). As a forest margin relict, *Virgilia* is closely associated with afromontane forest. The estimated divergence time of 20,000 to 180,000 years agrees with the last interglacial of the late pleistocene (some 100,000 years ago) when forests must have extended over much of the coastal lowlands of the southern Cape region (Coetzee *et al.*, 1983). The subsequent glacial (80,000 to 30,000 years ago) with its resultant drier climate, may have resulted in the gradual isolation of afromontane forest (and *Virgilia*) to small isolated river valleys as we see today.

Despite morphological differences, the results of the present study indicate that *V. oroboides* possesses a sufficient amount of genetic variation to allow it to adapt to environmental changes or to be used in selection programmes. The amount of variation obtained in the present study is also not unexpected when compared to values reported for the majority of species from the same family, and to plant species with similar life history characteristics (see Van der Bank *et al.*, 1995).

An association was found between genetic and geographic distance between populations. Values of A reduced progressively from the eastern population sampled (Tradouws Pass) towards the western geographical regions (Table 3). This was also true for P and $\bar{H}$ values, except for Kirstenbosch where slightly higher values were found compared to that of the Betty's Bay population. The pattern of allozyme variation (Tables 2 and 3) suggests that *V. oroboides* has undergone gradual genetic divergence following isolation of populations. This pattern of divergence and the associated loss of alleles (explicitly shown in Fig. 2) is most concordant with an allopatric mode of speciation as suggested in the introduction. These relationships are also evident from the genotypic distance index values (Table 3) of Nei (1978) and Cavalli-Sforza and Edwards (1967). The relatively low value for

the mean genotypic distance (Nei, 1978 = 0.0185) suggests that little genetic differentiation has occurred since these populations shared a common ancestor, and there are indeed only subtle morphological differences between populations. Differences (at the population level) in flower colour, branching pattern, size and shape of bracts, vestiture density and flowering time are probably all genetically fixed. A provenance trial of 18 populations of all three taxa of *Virgilia*, each represented by seven replications in a randomised block design, was planted in Stellenbosch in August 1981 (Van Wyk, 1982). Despite poor survival, it was possible to show that vestiture density, flower colour and flowering time were not influenced by environmental conditions and that these characters were exactly the same in the cultivated progeny as in the parent trees from which the seeds were originally collected. Long-lived species maintain high levels of genetic variation within their populations (Hamrich, 1979; Hamrich *et al.*, 1979), presumably because of large numbers of generations present in any given population (Levin, 1978). In *Virgilia* however, each generation is killed off by recurrent fires, and the population regenerates from seeds.

Recent isolation is considered to be the most likely explanation for the low intraspecific genetic divergence encountered in the populations studied. This is supported by the divergence time estimated (average = 0.093 million years ago). We conclude that the level of genetic diversity maintained within *V. oroboides* populations, and the level of population divergence found, is strongly influenced by its human and insect associated mode of gene flow via seed dispersal and cross-fertilisation, respectively. It would be interesting to see if allozyme data support the presumed relationships between the species and subspecies of *Virgilia* as reflected in a recent taxonomic revision based on morphological characters (Van Wyk, 1986). It was suggested that *V. oroboides* ssp. *ferruginea* may have resulted from introgression between typical *V. oroboides* and *V. divaricata*. In *V. oroboides* ssp. *ferruginea*, vegetative characters of *V. oroboides* are combined with reproductive characters of *V. divaricata* (Van Wyk, 1986), but alkaloid data (Greinwald *et al.*, 1989; Veen *et al.*, 1991) did not support a hybrid origin for this subspecies. It may be interesting to evaluate allozyme data at this higher taxonomic level.

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