Genetic variation in an endangered cedar (*Widdringtonia cedarbergensis*) versus two congeneric species

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Widdringtonia cedarbergensis is a southern African conifer species under threat of extinction. This study explored the genetic status of the species to assess whether loss of genetic variation may be contributing to population declines. *Widdringtonia nodiflora* and *W. schwarzii* were used as benchmarks against which to compare genetic diversity in *W. cedarbergensis*. Isozyme electrophoresis was used to resolve seventeen isozyme loci in seedlings of the three species. Genetic diversity was greatest in *W. nodiflora*, followed by *W. cedarbergensis* and *W. schwarzii*. There is no evidence that *W. cedarbergensis* has undergone a genetic bottleneck relative to its sister species. Patterns of genetic variation varied between species with most of the variation occurring within populations of *W. cedarbergensis*, between populations of *W. schwarzii*, and within population 'neighbourhoods' of the more widespread *W. nodiflora*. The isozyme data indicate inbreeding, probably due to self-pollination, in *W. cedarbergensis* and *W. nodiflora*. Populations of *W. schwarzii* were outbreeding. Selfing in *W. cedarbergensis* may be caused by a change in population density from dense to sparse stands with potentially deleterious genetic consequences.

Keywords: Conifers, endangered, fragmentation, population genetics, *Widdringtonia*

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Introduction

Estimating levels and patterns of genetic diversity within threatened species has become an important aspect of conservation biology. As species become increasingly fragmented, sub-populations become smaller and more isolated and as a result, begin to face demographic and genetic risks (Barrett & Kohn 1991; Lacy 1992) resulting in a loss of genetic variation. A high level of genetic variation can provide insurance against extinction, while a low level, sometimes caused by population bottlenecks, is likely to lead to a greater risk of extinction. Inbreeding depression in small, isolated populations can result in loss of fitness which, in turn, influences population demography (Ellstrand & Elam 1991). The theoretical importance of genetic principles in conservation has been widely accepted. However empirical evidence for the importance of genetics in conserving species, especially plant species, is still very limited (Young *et al.* 1996). We studied the possible importance of genetic processes in the conservation of the endangered conifer, *Widdringtonia cedarbergensis*.

Widdringtonia cedarbergensis (the Clanwilliam cedar) is a tree species endemic to the Cederberg in the western Cape. The species is rated as endangered in the most recent South African Red Data Book (Hilton-Taylor 1996). Though the cedar occurs in a landscape dominated by fire-prone fynbos shrublands, it seems poorly adapted to prevailing fire regimes. Mature trees are easily killed by fire, there is no canopy-stored seedbank, sapling growth rates are relatively slow and the trees take longer than any co-occurring fynbos shrub to reach maturity (Manders 1985). This set of traits has led to a marked decline in the population in the prevailing fynbos fire regime (Manders 1986; Brown *et al.* 1991). Special fire management procedures have had to be devised for the cedar in a 'cedar reserve'. A replanting scheme has also been initiated using seeds which have been collected predominantly from a cedar plantation (Mustart *et al.* 1995).

One explanation for the apparent poor adaptation of cedars to contemporary fire regimes may be that it has passed through a population bottleneck resulting in a lack of genetic variation on which natural selection might act. Another possibility is that the population structure has changed from a relatively dense fire-proof woodland to the current structure of scattered trees in a matrix of fynbos. The species is thought to have been heavily utilised by intensive logging in the last two centuries and this may have contributed to its current fragmented population structure (Manders 1986).

We studied genetic variation and structure in *W. cedarbergensis*, (1) to test whether it had particularly low levels of genetic variation relative to two congeneric species, *W. nodiflora* and *W. schwarzii* indicative of a genetic bottleneck; (2) to determine patterns of genetic variation in the three species in relation to their life history attributes and 3) to determine the genetic relationships of the Cederberg populations and their bearing on selection of seeds for the replanting programme.

W. cedarbergensis, *W. nodiflora* and *W. schwarzii*, differ in their biology and distribution. These differences are outlined in Table 1. Differences in reproductive biology and life history attributes are known to influence patterns of genetic variation (e.g. Hamrick & Godt 1990). We therefore expected different population genetic structures regardless of possible bottleneck effects. If *W. cedarbergensis* is experiencing a bottleneck effect, then levels of genetic diversity should be lower than those of the other two species.

All three species are wind-pollinated and should all, therefore, be panmictic to some extent. Gene flow could possibly differ among species because of their different seed dispersal syndromes, fire survival strategies and geographic distribution.

W. cedarbergensis:- Gene flow between populations may be reduced because of the very poorly dispersed heavy seeds (Manders 1987). *W. cedarbergensis* is a geographically restricted species and therefore relatively low levels of polymorphism were expected. A general tendency has been shown for widespread species to have a higher degree of genetic polymorphism than narrowly restricted species (Karron *et al.* 1988), although other

Table 1 The biology and distribution of three species of *Widdringtonia*

Species	Regeneration Mode	Seed Morphology	Serotiny Level	Distribution
W. cedarbergensis	Non-sprouter	Large, heavy	Nonserotinous	Cederberg
W. nodiflora	Resprouter	Light, winged	Highly serotinous	Cape to Malawi
W. shwarzii	Non-sprouter	Light, winged	Mildly serotinous	Baviaanskloof, Kouga

ecological factors may confound this trend (Loveless & Hamrick 1984).

W. nodiflora: We expected gene flow between populations to be high since seeds are light and winged, enabling long-distance dispersal. However, *W. nodiflora* is distributed along most mountain ranges up the east coast to central Africa as far as Malawi and distances between these mountain ranges could present a barrier to gene flow. We therefore expected patterns of population differentiation to be influenced by these distances. Since it is a widespread species and displays a high degree of morphological variation (Marsh 1966; Pauw 1992), we expected high levels of genetic differentiation among different populations in *W. nodiflora*. *W. nodiflora* resprouts after fire and therefore adults persist for many generations. This should lead to an accumulation of heterozygotes within populations. Resprouting should affect population differentiation in that the establishment of individuals and populations would be a rare event since the probability of a new cohort of seedlings being eliminated by fire is high.

W. schwarzii: Gene flow between populations is potentially high owing to long-distance dispersal of light, winged seeds. Outcrossing between populations could be hindered by the confinement of populations to deep, narrow, relatively fire-proof kloofs in the Baviaanskloof and Kouga mountains. We therefore expected relatively high levels of genetic differentiation among populations since they are discrete and widely separated by intervening ridges. *W. schwarzii* is also a geographically restricted species and we therefore expected relatively low levels of genetic diversity.

W. cedarbergensis and *W. schwarzii* should show similar levels of genetic diversity, notwithstanding the effects of recent fragmentation, since both have the same limited distribution and similar biologies barring differences in seed dispersal.

Materials and methods

Sampling

Populations of *W. cedarbergensis*, *W. nodiflora* and *W. schwarzii* were visited and sampled for cones. Populations from most of the range of *W. cedarbergensis* was sampled. *W. schwarzii* grows on cliff faces and steep ravines and, because of difficulties of access, only three populations were sampled. *W. nodiflora* is a widespread species. Sampling was concentrated on populations occurring in the Cape Floristic Region, where conditions are most similar to its congeners, but a single population from the Natal Drakensberg was also included.

Seven populations of *W. cedarbergensis* were sampled [Hoogvertoon (SB), Duiwelsgat (DG), Welbedacht (WB), Crystal Pools (CPS), Middelberg (MB), Krakadouw Kloof (KK) and Heuningvlei (KD)]; seven populations of *W. nodiflora* [Kirstenbosch (KB), Orangekloof (OK), Bainskloof pass (BKR), Baviaanskloof in Bainskloof (BKV), Steenbras river mouth (SBR), Betty's Bay (BBAY) and Cathedral Peak (CPK)]; and three populations of *W. schwarzii* [Doringkloof (DK), Sandvlakte (SV) and Nuwekloof (NK)] (Figure 1). The plantation of *W. cedarbergensis* used as the main seed source for the replanting programme is denoted as MB, not to be confused with an additional *W. cedarbergensis* plantation, KD.

Four to five cones from 30 to 40 randomly positioned trees per population were sampled. Cones were oven dried at 60°C to release the seeds which were then germinated in a germination chamber with temperatures alternating between 10°C and 20°C on a 12 hour cycle. The germlings were allowed to develop until the secondary shoot bud was detectable. Isozymes for one seedling per tree were analysed. The seedlings were then crushed in a chilled mortar and pestle using vegetative extraction buffer 1 from Cheliak and Pitel (1984). Five filter paper wicks, which were cut to the size of 3 mm × 12 mm from no. 4 Whatmann filter paper, were saturated in the extract and placed in an Eppindorff tube which was directly transferred to a –20°C freezer. The isozymes were separated on 12% starch gels which were 10 mm thick using the enzyme/gel buffer combinations outlined in Conkle *et al.* (1982). Before each gel run, the wicks were inserted between the anodal and cathodal sections of the gels.

The gels were left to run for 5 hours at 60 mA and then sliced horizontally 2 mm thick. The following enzyme systems were stained using staining procedures outlined in Conkle *et al.* (1982): malate dehydrogenase (Mdh), shikimic acid dehydrogenase (Sdh), isocitrate dehydrogenase (Idh), glucose-6-phosphate dehydrogenase (G6pdh), glutamate dehydrogenase (Gdh), superoxide dismutase (Sod), diaphorase (Dia), aspartate aminotransferase (Aat), acid phosphatase (Aph), menadione reductase (Mnr), peroxidase (Per), malic enzyme (Me), phosphoglucose isomerase (Pgi), phosphoglucomutase (Pgm), leucine amino peptidase (Lap), α-esterase (α-Est), β-esterase (β-Est), fluorescent esterase (Flest) and aconitase (Acon).

The gels were interpreted and scored. Loci were labelled in ascending order from 1 to 3 from fastest (migrating furthest in the gel towards the cathodal end) to slowest (migrating least in the gel towards the cathodal end). Alleles were labelled from A to Z in ascending order from fastest to slowest.

Data analysis

For each population for which allozyme data were collected, genotype arrays were analysed using the Biosys-1 package (Swofford & Selander 1989). Allele frequencies were calculated and used in conjunction with the genotype data to calculate mean genetic diversity estimates including: number of alleles per locus (A), % of polymorphic loci (P), mean proportion observed heterozygosity (H_o) and mean proportion expected panmictic heterozygosity (H_e). Levels of inbreeding within populations were estimated through comparing H_o and H_e. H_e is an expected genotypic frequency calculated by using a binomial expansion of the allele frequencies. This binomial expansion is called the Hardy-Weinberg principle (Nei 1978). If observed levels of heterozygosity conform with expected levels of heterozygosity, the population is said to be in Hardy-Weinberg equilibrium. The inbreeding coefficient, F, can be used to compare levels of inbreeding between subpopulations. F is defined as the probability that two alleles at a genetic locus in the inbred individual are descended from a single gene in a single ancestor shared by the parents (Wright 1969) and is characterised by the formula $F = (H_e - H_o)/H_e$ where H_e is the expected heterozygosity at a locus and H_o is the observed heterozygosity at a locus.

The levels and distribution of genetic diversity were calculated using Wright's fixation indices (Wright 1965) These statistics were used to describe three levels of genetic interaction. The basic formula used in Biosys-1 is: $1 - F_{it} = (1 - F_{is})(1 - F_{st})$. F_{is} represents

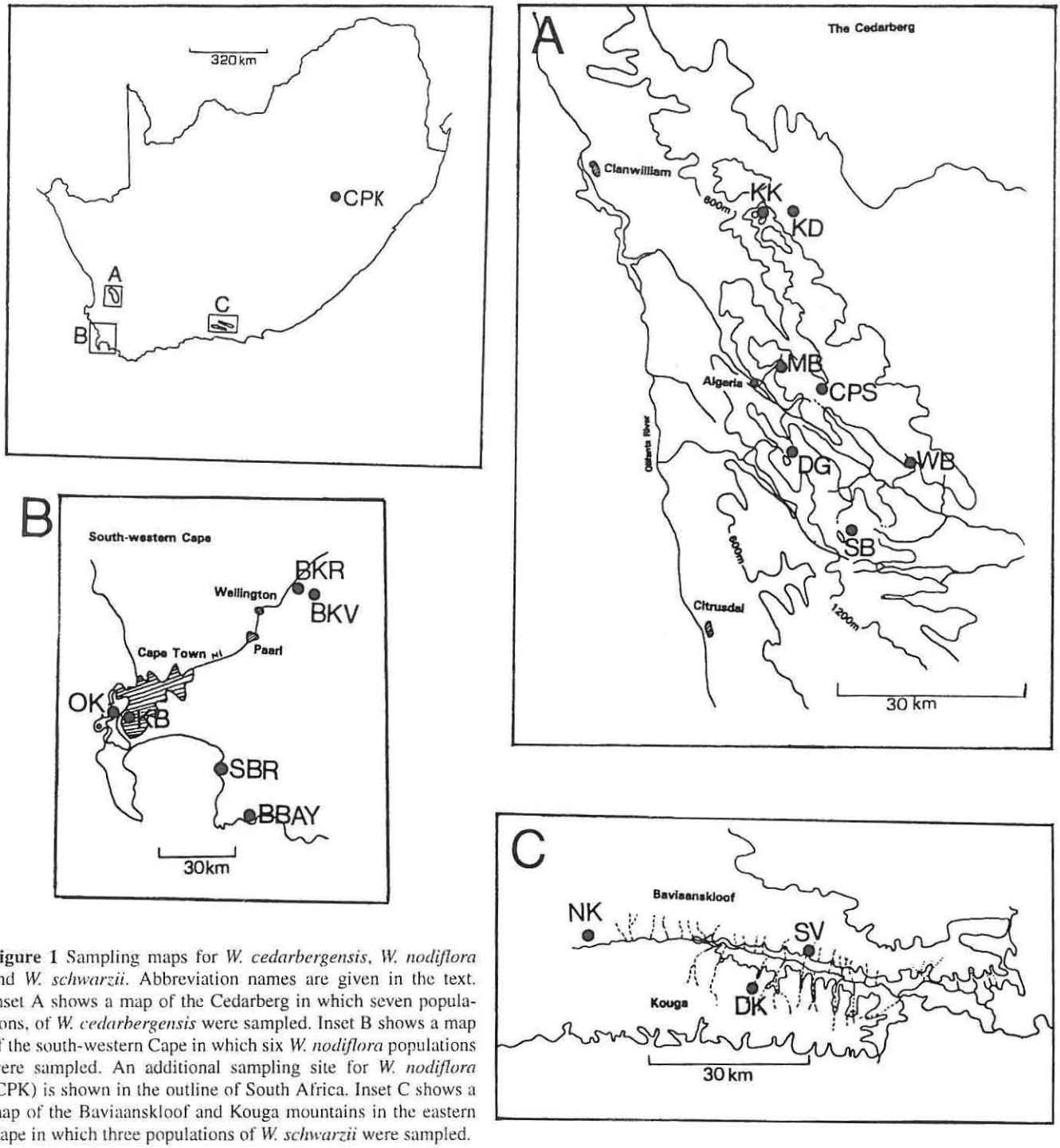

Figure 1 Sampling maps for *W. cedarbergensis*, *W. nodiflora* and *W. schwarzii*. Abbreviation names are given in the text. Inset A shows a map of the Cedarberg in which seven populations, of *W. cedarbergensis* were sampled. Inset B shows a map of the south-western Cape in which six *W. nodiflora* populations were sampled. An additional sampling site for *W. nodiflora* (CPK) is shown in the outline of South Africa. Inset C shows a map of the Baviaanskloof and Kouga mountains in the eastern Cape in which three populations of *W. schwarzii* were sampled.

the level of genetic interaction between individuals within the same deme or tree cluster. A positive F_{IS} is associated with deficiencies of heterozygotes and suggests inbreeding while a negative F_{IS} suggests too many heterozygotes relative to Hardy-Weinberg equilibrium (Linhart *et al.* 1981). F_{ST} represents the correlation between random gametes within a given deme relative to gametes within the whole population. This value is used to determine the amount of differentiation between sub-populations and is an indirect measure of gene flow (high differentiation among sub-populations implies low gene flow). F_{IT} represents the correlation between uniting gametes, and therefore the fixation index, within the whole population.

Genetic distance and cluster analysis

Nei's (1978) genetic identity (I) was used to describe the extent of genetic relationships between populations of each species. Pairwise comparisons were made and the identity values (I) obtained and translated into a UPGMA (unweighted pair group method with arithmetic averaging) cluster analysis. The UPGMA algorithm used in Biosys-1 is described in Sneath & Sokal (1973). Mantel's *t*-test was used with the aid of NTSYS - pc (Rohlf 1993), to compare cophenetic genetic identity and geographic distance matrices.

Results

Gene frequencies

Allele frequencies for 19 enzyme loci were calculated. Five of these loci were found to be polymorphic in *W. cedarbergensis* (Table 2a), seven were found to be polymorphic for *W. nodiflora* (Table 2b) and four were found to be polymorphic for *W. schwarzii* (Table 2c). Allele frequencies among different populations of *W.*

Table 2 Allele frequencies obtained at polymorphic loci for (a) *W. cedarbergensis* (b) *W. nodiflora* and (c) *W. schwarzii* (see text for population name abbreviations)

Locus	Allele	WB	SB	DG	CPS	MB	KD	KK
				(a) *W. cedarbergensis*				
PGI-2	A	0.714	0.859	0.444	0.431	0.632	0.357	0.500
	B	0.286	0.141	0.556	0.519	0.368	0.643	0.500
SDH-2	A	0.637	0.833	0.280	0.578	0.750	0.528	0.813
	B	0.363	0.167	0.720	0.422	0.250	0.472	0.188
IDH-1	A	0.033	0.225	0.206	0.224	0.111	0.125	0.000
	B	0.678	0.650	0.676	0.466	0.833	0.719	1.000
	C	0.289	0.125	0.118	0.310	0.056	0.156	0.000
LAP-2	A	0.838	0.261	0.680	0.774	0.947	0.773	0.983
	B	0.162	0.717	0.320	0.226	0.053	0.227	0.017
	C	0.000	0.022	0.000	0.000	0.000	0.000	0.000
AAT-2	A	0.750	0.235	0.444	0.375	0.900	0.545	1.000
	B	0.250	0.765	0.556	0.625	0.100	0.455	0.000
				(b) *W. nodiflora*				
Locus	Allele	KB	OK	BKR	BKV	SBR	BBAY	CPK
PGI-1	A	1.000	0.786	0.094	0.031	1.000	1.000	0.667
	B	0.000	0.214	0.906	0.969	0.000	0.000	0.333
PGI-2	A	0.938	0.778	0.922	0.968	0.932	1.000	0.833
	B	0.063	0.204	0.078	0.032	0.045	0.000	0.167
	C	0.000	0.000	0.000	0.000	0.023	0.000	0.000
	D	0.000	0.019	0.000	0.000	0.000	0.000	0.000
SDH-1	A	0.576	0.707	0.468	0.579	0.774	0.944	0.559
	B	0.424	0.259	0.532	0.421	0.210	0.056	0.441
	C	0.000	0.000	0.000	0.000	0.016	0.000	0.000
	D	0.000	0.034	0.000	0.000	0.000	0.000	0.000
SDH-2	A	0.000	0.000	0.000	0.000	0.000	0.000	1.000
	B	0.076	0.000	0.032	0.105	0.000	0.000	0.000
	C	0.924	1.000	0.968	0.895	1.000	1.000	0.000
IDH-1	A	0.188	0.231	0.188	0.088	0.679	0.778	0.059
	B	0.719	0.692	0.438	0.500	0.321	0.222	0.500
	C	0.094	0.077	0.375	0.412	0.000	0.000	0.441
MDH-3	A	0.200	0.400	0.235	0.286	0.294	0.500	1.000
	B	0.433	0.233	0.559	0.543	0.676	0.500	0.000
	C	0.367	0.367	0.176	0.171	0.029	0.000	0.000
	D	0.000	0.000	0.029	0.000	0.000	0.000	0.000
LAP-1	A	0.061	0.000	0.000	0.000	0.094	0.000	0.000
	B	0.000	0.089	0.071	0.000	0.078	0.000	0.000
	C	0.000	0.000	0.186	0.071	0.000	0.000	0.000
	D	0.742	0.804	0.586	0.914	0.672	1.000	1.000
	E	0.197	0.107	0.114	0.014	0.156	0.000	0.000
	F	0.000	0.000	0.043	0.000	0.000	0.000	0.000
				(c) *W. schwarzii*				
Locus	Allele	SV	DK	NKA				
PGI-2	A	0.222	0.050	0.000				
	B	0.778	0.900	0.850				
	C	0.000	0.050	0.150				
IDH-1	A	0.350	0.600	0.700				
	B	0.650	0.400	0.300				
MDH-3	A	0.500	0.600	0.550				
	B	0.500	0.400	0.450				
LAP-2	A	0.900	0.850	0.700				
	B	0.100	0.150	0.300				

schwarzii were substantially more uniform than among populations of *W. cedarbergensis* and *W. nodiflora*. Major allelic differences between populations of *W. cedarbergensis* included (i) an extra slow allele at the Lap-2 locus in SB and (ii) monomorphism at Idh-1 and Aat-2 loci in KK (Table 2a). Allelic differences between populations of *W. nodiflora* included (i) the presence of a unique fast A-allele at Sdh-2 in CPK, (ii) the presence of a unique C-allele at Sdh-1 in SBR, and (iii) the presence of a unique D-allele at Mdh-3 in BKR. Many absences of alleles were found among populations of *W. nodiflora*. Extreme cases were BBAY which had 16 missing alleles in total and CPK which had 14 missing alleles (Table 2b). In *W. schwarzii*, only SV was unique with an absent C-allele at PGI-2 (Table 2c).

Estimates of genetic diversity

The number of alleles per locus (A) were calculated for each population within each species (Table 3). This calculation included monomorphic loci since the extreme cases where all loci were monomorphic would have A = 1. The mean number of alleles per locus averaged over all populations within each species showed populations of *W. nodiflora* to have a much higher allelic diversity than populations of *W. cedarbergensis* and *W. schwarzii*. *W. schwarzii*, in particular, had the lowest level of allelic variation at each locus. Populations of *W. nodiflora* vary greatly with respect to A. BBAY had the least allelic diversity and BKR had the highest levels of allelic diversity, whereas populations of *W. cedarbergensis* were mostly uniform with respect to A except for the small isolated population, KK, which showed low levels of allelic diversity. One population of *W. schwarzii*, DK, had a higher allelic diversity than the other two. Compared with other studies of gymnosperms, all three species of *Widdringtonia* had low values for A (mean A = 1.93 for gymnosperms in Hamrick & Godt 1990).

Table 3 Estimates of genetic diversity in populations of (a) *W. cedarbergensis*, (b) *W. nodiflora* and (c) *W. schwarzii*. N = sample size; A = average number of alleles per locus; P = percentage polymorphic loci; H_o = mean observed frequency of heterozygotes; H_e = mean expected frequency of heterozygotes; F = fixation index. Values in parentheses indicate standard errors

Population	N	A	P	H_o	H_e	F
			(a) *W. cedarbergensis*			
WB	36	1.32	29.4	0.28	0.40	0.302 (0.21)
SB	23	1.36	29.4	0.26	0.37	0.250 (0.34)
DG	23	1.32	29.4	0.37	0.47	0.209 (0.44)
CPS	36	1.32	29.4	0.37	0.50	0.250 (0.15)
MB	14	1.32	29.4	0.27	0.31	0.042 (0.32)
KD	17	1.32	29.4	0.35	0.47	0.217 (0.18)
KK	19	1.16	17.7	0.18	0.17	-0.083 (0.11)
Mean		1.30	27.7	0.30	0.39	0.170
			(b) *W. nodiflora*			
KB	23	1.47	35.3	0.29	0.41	0.233 (0.39)
OK	25	1.57	35.3	0.29	0.44	0.325 (0.12)
BKR	28	1.68	41.2	0.28	0.40	0.301 (0.41)
BKV	23	1.53	41.2	0.21	0.32	0.098 (0.31)
SBR	24	1.52	29.4	0.24	0.39	0.278 (0.30)
BBAY	9	1.16	17.7	0.22	0.34	0.210(0.19)
CPK	23	1.26	23.5	0.22	0.49	0.245 (0.47)
Mean		1.46	31.9	0.25	0.40	0.241
			(c) *W. schwarzii*			
SV	20	1.21	23.5	0.33	0.40	0.097 (0.25)
DK	10	1.26	23.5	0.38	0.37	0.007 (0 46)
NK	10	1.21	23.5	0.45	0.42	-0.029 (0.61)
Mean		1.23	23.5	0.39	0.40	0.025

Two measures of heterozygosity are given in Table 3. H_o gives the mean proportion observed heterozygosity for all polymorphic loci within each population and H_e gives the mean proportion expected heterozygosity within each population. The mean H_o for all populations is highest in *W. schwarzii* and lowest in *W. nodiflora*.

Mean observed and expected levels of heterozygosity varied among populations of all three species although only populations of *W. schwarzii* conformed to Hardy-Weinberg equilibrium (Table 3). Populations in both *W. cedarbergensis* and *W. nodiflora* showed departure from random mating, with few exceptions. The fixation index (F) gives an estimate of the difference between observed and expected heterozygosity. F ranges from 0 to 1, with values of 0 indicating a panmictic (outcrossing) population and values of 1 indicating selfing. The mean fixation indices of all populations within all species show that populations of *W. nodiflora* deviated from Hardy-Weinberg expectations substantially more than populations of *W. cedarbergensis* and *W. schwarzii* (Table 3). In *W. cedarbergensis*, the most inbred populations seem to be WB and SB where fixation indices were fairly high. The plantation, MB, was the only 'population' which seems to be outbreeding. KK would also appear to be outbred although this result may be a function of sampling error since KK is monomorphic for an additional two loci. In *W. nodiflora*, the most inbred populations seem to be OK, BKR and SBR where fixation indices were particularly high. BKV was the only population with a low fixation index.

F-statistics

F_{is} values are comparable to the fixation index and measure the degree of selfing. F_{is} was much lower in *W. schwarzii* than in the other two species (Table 4). F_{is} was high in *W. cedarbergensis*, but especially high in *W. nodiflora*. Similar trends can be seen for F_{it} and F_{st}. The F_{st} for *W. nodiflora* is so high that it approaches values for selfing plants (G_{st} = 0.510 in Hamrick & Godt 1990) implying extremely low levels of gene flow between populations. *W. schwarzii* was the only species within the genus that has an F_{st} value close to that found for most gymnosperms which generally have high levels of gene flow and little population differentiation (mean G_{st} for gymnosperms = 0.068, Hamrick & Godt 1990).

Genetic distance and cluster analysis

The results of the pairwise comparisons of genetic distance between populations using Nei's unbiased genetic distance are presented graphically using a UPGMA cluster analysis (Figure 2). Mantel's *t*-test shows there was no relationship between geographic proximity of populations (see Figure 1) and genetic

Table 4 Wright's *F*-statistics for levels of gene flow within and between populations of (a) *W. cedarbergensis*. (b) *W. nodiflora* and (c) *W. schwarzii*.

Locus	F_{is}	F_{it}	F_{st}
	(a) *W. cedarbergensis*		
Pgi-2	0.273	0.350	0.106
Sdh-2	0.079	0.206	0.138
Idh-1	0.390	0.447	0.094
Lap-2	0.426	0.573	0.255
Aat-1	-0.136	0.193	0.289
Mean	0.206	0.354	0.176
	(b) *W. nodiflora*		
Pgi-1	0.656	0.890	0.681
Pgi-2	0.066	0.123	0.060
Sdh-1	-0.157	-0.039	0.102
Sdh-2	0.468	0.899	0.811
Idh-1	0.364	0.500	0.214
Lap-2	0.519	0.571	0.109
Mdh-3	0.315	0.461	0.213
Mean	0.364	0.498	0.313
	(c) *W. schwarzii*		
Pgi-2	0.335	0.372	0.056
Idh-1	0.336	0.394	0.088
Lap-2	-0.053	-0.002	0.048
Mdh-3	-0.559	-0.549	0.007
Mean	-0.015	0.054	0.050

identity (see Figure 2) in *W. cedarbergensis* ($t = -0.37$; $p = 0.356$). SB was the most unique population. The Middelberg plantation, MB, which is used as the principle seed source for replanting programmes, was identical to WB and the KD plantation was most related to DG and CPS.

In *W. nodiflora*, there was a strong relationship between genetic identity and geographic proximity of populations (Mantel's *t*-test: $t = -2.95$; $p = 0.002$). The cluster analysis shows that populations of *W. nodiflora* situated close together, for example BKV and BKR, are as similar to each other as are populations of *W. schwarzii* and of *W. cedarbergensis*. Disjunctions in genetic similarity between mountain ranges were found in *W. nodiflora*. The population from the Natal Drakensberg, CPK, was an outlier to the Cape sample.

In *W. schwarzii*, all three populations were genetically very similar although NK and DK form a separate cluster. With such a small sample size it was impossible to test genetic and geographic distances for statistical signifcance.

Discussion

Levels of genetlc polymorphism

Narrowly endemic species are generally known to be genetically depauperate. This has been shown in case studies for plants such as the narrowly endemic *Bensoniella oregona* (Saxifragaceae) (Soltis *et al.* 1992), and the rare *Eucalyptus pulverentula* (Peters *et al.* 1990). Two extreme endemics, *Pedicularis furbishae* (Waller *et al.* 1987) and *Pinus torreyana* (Ledig & Conkle 1983) have been shown to have no variation at the loci examined (although one population of *Pinus torreyana* was polymorphic at 3.4% of the loci). Conversely, widespread species are generally known to be more variable than their more endemic congeners (Loveless & Hamrick 1984; Hamrick & Godt 1990; Karron *et al.* 1988). This general trend holds true for *Widdringtonia*. *W. nodiflora* was the most widespread species of all and has the highest allelic diversity and genetic polymorphism. The differences in levels of genetic diversity between the two geographically restricted species were slight. This suggests that the poor adaptation of the Clanwilliam cedar to current ecological conditions cannot be attributed to lack of genetic variation due to a genetic bottleneck. Although *W. cedarbergensis* may have suffered severe recent reductions in numbers, genetic erosion would be delayed by residual heterozygosity in adult trees, many of which have survived for centuries. In comparison with other narrowly restricted conifers, *W. cedarbergensis* and *W. schwarzii* certainly have higher levels of polymorphism and allelic diversity than has been found for the rare *Pinus torreyana* (Ledig & Conkle 1983).

There is sufficient evidence to suggest that these two components of diversity; polymorphism and allelic diversity; are more reliable indicators of the effects of population bottlenecks than are mean observed and expected proportions of heterozygosity. This has been shown mathematically by Nei *et al.* (1975), in computer simulations by Lacy (1987) and by Leberg (1992, 1993) in a semi-natural experiment on mosquitofishes. The life history of *Widdringtonia* is very different to that of a

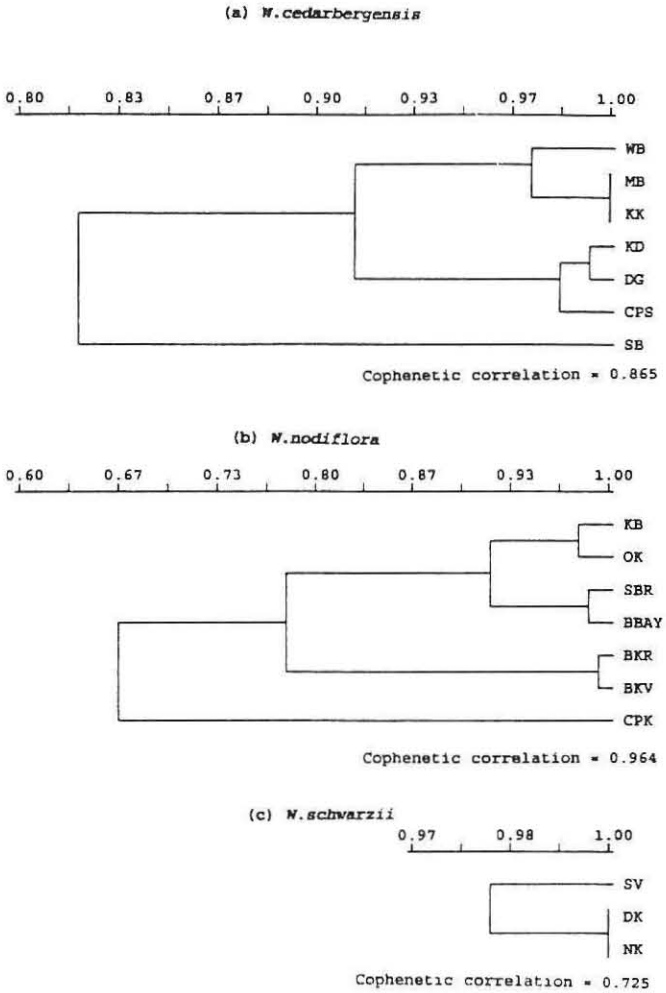

Figure 2 Dendrogram based on a UPGMA cluster analysis using Nei's (1978) genetic identity (I) calculated from the allelic frequencies of populations, showing relationships between populations of (a) *W.cedarbergensis*, (b) *W.nodiflora* and (c) *W.schwarzii*. The scale indicates I, genetic identity, ranging from O (no relationship) to 1 (identical).

mosquitofish which has a generation time of 56 days (Leberg 1993). However, average heterozygosity depends not only on the size of the bottleneck but also on the rate of population growth as the population recovers (Nei *et al.* 1975). Average number of alleles per locus, on the other hand, is profoundly affected by bottleneck size but not so much by population growth (Nei *et al.* 1975). The finding that *W. nodiflora*, the most abundant and widespread species within the genus has the lowest levels of heterozygosity, suggests that, in this case at least, heterozygosity is an unreliable indicator of the effects of bottlenecks in a between-species comparison.

Organisation of genetic variation

Patterns of genetic variation in the three species of *Widdringtonia* were indicated by Wright's *F*-statistics, observed and expected heterozygosity, and by patterns of genetic relatedness between populations. High F_{is} values and heterozygote deficiencies in *W. cedarbergensis* and *W. nodiflora* suggest the possibility of either inbreeding or the Wahlund effect due to high levels of population sub-structuring. Given the biology and ecology of these two species, these two factors need not mutually exclude each other in determining patterns of sub-structuring within populations.

The relatively high levels of inbreeding in the Clanwilliam cedar may be due to self pollination. Lack of pollen movement between trees may be caused by population level fragmentation with the majority of trees restricted to rocky outcrops in a sea of flammable fynbos and with large gaps between trees. Evidence for lowered outcrossing rates have been found in low density stands of ponderosa pine, compared with high density stands, as a result of inefficient pollen movement (Farris & Mitton 1984). If selfing is occurring as a result of the thinning of trees, then dense populations of cedars should be the most out-crossed. This appeared to be the case since *F*-coefficients for the large plantation (MB), with high tree densities, were very low indicating high out-crossing rates.

F_{st} values indicated high levels of population differentiation in *W. cedarbergensis* and *W. nodiflora* but showed *W. schwarzii* to be panmictic. Levels of population relatedness showed that *W. nodiflora* had a metapopulation that was structured into population neighbourhoods where populations from the same mountain range were highly related to populations from distant mountain ranges. *W. cedarbergensis*, on the other hand, had an unstructured metapopulation where neighbouring populations were not necessarily more related than more distant populations. The high level of population differentiation in *W. nodiflora* may to some extent be attributed to the effects of fire as predicted at the outset of the study but the cluster analysis shows that distance is a major barrier to gene flow since neighbouring populations of *W. nodiflora* are as related as populations of the highly panmictic *W. schwarzii*. Gene flow between populations of *W. nodiflora* is therefore efficient at close range. Population differentiation in *W. cedarbergensis* can be attributed to lack of gene flow within populations caused by poor seed dispersal and lack of pollen movement. This indicates the vulnerability of the Clanwilliam cedar's population genetic structure to fragmentation.

The genetic relationships between populations strengthens evidence for a historically more continuous distribution of *W. cedarbergensis*. Geographic proximity of populations was not correlated with genetic relatedness which implies that all the populations of *W. cedarbergensis* were once part of a greater panmictic population with a high degree of relatedness. As the metapopulation became increasingly fragmented, low levels of gene flow between the various sub-populations may have led to the genetic differentiation of the smaller sub-populations as a result of selfing. The seed source plantation at Middelberg (MB) has affinities with a small northern population (KK) and with a population included in the Cedar preservation area (WB).

Conclusion

Our electrophoretic comparison of genetic variation and structure in three *Widdringtonia* species showed little difference between the threatened *W. cedarbergensis* and the geographically restricted *W. schwarzii*. In long-lived trees such as these, a population bottleneck would be unlikely to have genetic effects except on timespans of millennia. Our data does provide some support for a more continuous distribution of the Clanwilliam cedar in the past. The current fragmented population structure may be leading to increased levels of selfing as trees become more distant from one another. The relatively high levels of inbreeding may be causing inbreeding depression. In conifers, this can be expressed in reduced seed production, poorer germination rates and reduced seedling growth (Charlesworth & Charlesworth 1987). Inbreeding depression due to fragmentation of cedar populations could therefore potentially contribute to population decline.

It is interesting to note the large differences in population genetic structure between the two non-sprouting species and the sprouting *W. nodiflora*. Genetically, this species behaves almost like an apomict with very little gene flow between populations. Genetic differences between sprouting and non-sprouting life histories have been ignored in previous reviews of life history effects on the distribution of genetic variation (e.g. Hamrick & Godt 1990). Further comparative studies are needed to establish the generality of the patterns reported here.

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