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A preliminary study of allozyme variation in three rare and restricted endemic *Barleria greenii* (Acanthaceae) populations

Tsepang Makholela [a], Herman van der Bank [b,*], Kevin Balkwill [a,1]

[a] *School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa*
[b] *Department of Zoology, Rand Afrikaans University, P.O. Box 524, Auckland Park 2006, South Africa*

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Abstract

Barleria greenii is a rare endemic taxon with an extremely restricted distribution area found only near Estcourt, South Africa. Three of eight known populations of *B. greenii*, representing a hierarchy from closely spaced to geographically distant sites, were studied by horizontal starch gel electrophoresis to assess the levels of genetic variation and to estimate the amount of genetic differentiation among populations. Sixteen enzyme coding loci provided interpretable results in all populations analysed, of which seven (43.8%) displayed polymorphism and nine (56.2%) displayed monoallelic gel banding patterns. Staining of some enzymes, which provided interpretable results in previous studies of other *Barleria* species, did not give any results in this study. The levels of genetic diversity measured in *B. greenii* are somewhat higher than the means for endemic populations, except for Population 1 and the average heterozygosity of Population 2a. Values of the mean number of alleles per loci increased progressively from the east (Population 1) to the west (Population 3) indicating clinal distribution. Short seed dispersal distance, parasitism and isolation by distance are considered to be the most likely explanations for the low genetic divergence encountered in the studied populations. The low genetic variability in *B. greenii* may also be a result of frequent or infrequent local extinction, possibly due to fires; therefore, fire regime should favour establishment of seedlings and resprouting of *B. greenii*.

Keywords: *Barleria*; Allozyme variation; Parasitism; Starch gel electrophoresis

* Corresponding author. Tel.: +27-11-489-2450; fax: +27-11-489-2286.
E-mail address: fhvdb@na.rau.ac.za (H. van der Bank).
[1] kevinb@gecko.biol.wits.ac.za

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1. Introduction

Barleria is a large genus of herbs and shrubs in the Acanthaceae, comprising approximately 300 species worldwide. The genus is distributed throughout the Old World tropics and sub-tropics, but with no representatives in Australia and only one in the New World. Few species in the genus are widespread, with most species showing a high degree of regional endemism (Balkwill et al., 1990). Endemism in many cases seems to be attributable to edaphic factors such as soil type. Approximately 45 of the 70 species are endemic to southern Africa, some with highly restricted distributions spanning only one or two-quarter degree squares (Balkwill et al., 1990). One such endemic is *B. greenii* M. & K. Balkwill which is listed as vulnerable in the Red Data book (Hilton-Taylor, 1996). It forms big shrubs that occur in large, dense and discrete populations that are restricted to black, shrink–swell clays above 1200 m; it is extremely localized and evidently has specific habitat requirements and should therefore be considered a rare species. Little is known about the genetic structure of the species and/or populations in this genus. The only published work is that of Van der Bank et al. (1999) in which molecular data was used to determine the possible origin of a species. Nothing further is known of the population genetics of the species in *Barleria*.

Studies of genetic variation in natural populations usually involve two basic questions. The first is concerned with describing levels of genetic variation maintained within populations or species. To approach this question, population geneticists have turned to biochemical techniques, primarily electrophoresis, to obtain estimates of genetic variation in plant and animal populations (Schonewald-Cox et al., 1983). These estimates are usually quantified in terms of the percentage of polymorphic loci per population, the average number of alleles per locus, or the mean number of heterozygous loci per population. The second question is concerned with the way in which genetic differentiation is partitioned among and within populations and it is of particular importance to the conservation of genetic resources.

In plant species, characteristics that should influence the distribution of genetic variation include the effective population size, the geographic distribution of the species, the primary mode of reproduction, the mating system, the seed dispersal mechanism, and the community type in which the species commonly occurs. Barriers to continual dispersal may be imposed by the following three factors: (1) geographic distance; distance alone may reduce the likelihood of movement of sufficient numbers of seeds or pollen among populations to prevent random events from affecting genetic divergence of populations (Wright, 1943; Mayr, 1970); (2) ecological distance; the presence of different habitat types between populations and or physical barriers imposed by geographic formations, such as rivers and mountains, may inhibit gene flow (Mayr, 1970); and (3) behavioural distance; social structuring of populations may inhibit successful entry of dispersing individuals into existing hierarchies even though movement among the units would otherwise be achieved easily (Selander et al., 1970; Daly, 1981; Chesser et al., 1982a,b). In addition biotic factors, such as parasites, often may have strong selective forces that affect fitness, differ-

ences in fitness and variation among populations. Management plans must consider the importance of these factors and their effects on the preservation of diversity in a dynamically evolving system (Schonewald-Cox et al., 1983).

Many of the activities of our society affect the genetic structure and dynamics of natural populations. For instance, the division of a population into smaller, semi-isolated units is a frequent result of human construction activities. Roads, dams, fences and buildings can all serve to isolate adjacent populations. These structures may restrict (and in some cases even eliminate) gene flow between populations. The consequence of such restricted gene flow is often that separate units diverge genetically.

The aim of this research was to elucidate the population genetic structure and breeding biology in *B. greenii*, to determine the levels of inbreeding and outbreeding within populations and the extent and patterns of gene flow between three populations. We need to determine if populations of *B. greenii* consist of a single random mating unit; or a series of small subpopulations (each largely isolated from each other—the stepping-stone or islands model); or a continuous population, but with individuals within it exchanging genes only with geographically proximate individuals (the isolation-by-distance model) (Baverstock and Moritz, 1996). A full understanding of population genetics and breeding biology is necessary to formulate biologically meaningful conservation management plans for this rare restricted endemic taxon.

2. Materials and methods

2.1. Plant material

B. greenii is a perennial, profusely branched woody shrub up to 1.8 m high or, if burnt regularly, forms very densely branched suffrutices up to 0.8 m high and 1.2 m in diameter (Balkwill et al., 1990). This species is known to occur in eight localities on three farms (Bosmans Riviers Poort, Selbourne and Van der Merwes Kraal) near Estcourt (Fig. 1). Of these, three sites were visited to collect samples for isozyme analysis. The first population was collected from the type locality on the farm Van der Merwes Kraal 972 and others from the farm Selbourne 1311 (Table 1). The geographical distances are 4.87 km between Populations 1 and 3, 3.24 km between Populations 1 and 2a, and 1.91 km between Populations 2a and 3.

At all sites, the plants occur in open, rocky areas on moderately sloping north-facing aspects, mostly between 1200 and 1260 m. The soil at all of the localities consists of very heavy, dense black clay strewn with doleritic rock. The plants grow at the interface of grassland and valley bushveld occurring in, or along, the borders of seasonal or perennial streams, drainage lines or boggy areas. Most plants grow between the rocks in full sun and those that grow in the shade are much less robust and have broader leaves (Balkwill et al., 1990).

In *B. greenii*, as in many members of the Acanthaceae, dispersal of seeds occurs ballistically (Bremekamp, 1926) and therefore takes place over short distances having

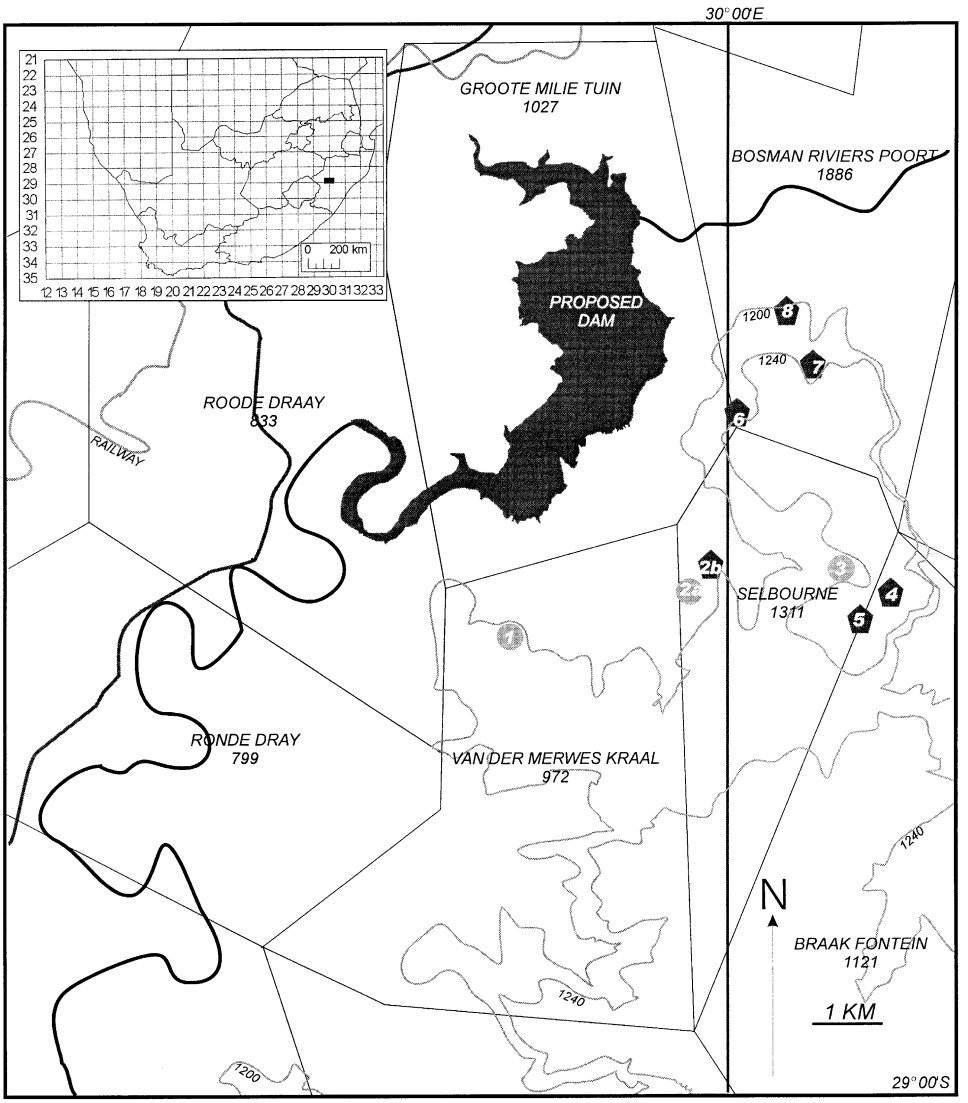

Fig. 1. Distribution map of *Barleria greenii*. Circles ● show populations sampled in this study and pentagons ⬟ show other known populations. Most populations occur between 1200 and 1240 m contours. These contours are marked on the map.

a profound effect on the distribution of the species. Long range dispersal to new suitable sites occurs rather rarely, but when it does occur, the seedlings mature and by the second year are able to produce offspring which grow close to their bases, and the population thus expands to fill the suitable habitat (Balkwill et al., 1990).

B. greenii flowers from mid- to late-summer, usually over a period of a few weeks, but if weather conditions are favourable, this may extend to a few months. The

Table 1
Sample collection sites for *Barleria greenii*

Population	Locality	Latitude	Longitude	Altitude (m)	Estimated area (m^2)	Density (samples/ha)
1	Estcourt on the farm Van der Merwes Kraal	28°56′35″S	29°58′10″E	1231	2297.14	139.3
2a	Estcourt on the farm Selbourne	28°56′10″S	29°59′40″E	1190	1655.05	193.3
3	Estcourt on the farm Selbourne	28°56′56″S	30°00′44″E	1211	984.96	324.9

attractive flowers range from pure white to dark pink with magenta streaks on the corrolla lobes. Individual plants consistently produce flowers of a particular colour, although sometimes with a slight variation relating to the age of the flowers. They emit a strong, sweet fragrance nocturnally and produce large quantities of nectar. Hawkmoths have been observed on flowers at night, but other species of moths seem not to be attracted to the flowers. Bumblebees are frequent daytime visitors to the flowers, but they are unlikely to be effective pollinators as they remove the nectar via a small slit made in the side of the corolla tube (Balkwill et al., 1990). Fruits are produced from early to late autumn.

2.2. Procedure

Leaf samples from 32 individuals of *B. greenii* were collected from each of three natural populations in May 2001 (Fig. 1). Three out of eight populations were selected to represent a hierarchy from closely spaced to geographically distant sites as suggested by Baverstock and Moritz (1996) in order to identify locally polymorphic markers as well as those with widespread variation. Population 1 was an outlier giving a measure for an isolated population (Fig. 1). Population 2a is close to Population 2b and to Population 3. Population 3 is towards the end of an East–West axis giving a measure of gene flow across one axis of the distribution of the species. In addition these populations were close to vehicle tracks, making it possible to place samples into a large liquid nitrogen cylinder immediately after picking leaves. Considering the difficulties of access to other populations the results of this preliminary study will give direction as to how the remaining populations should be sampled to provide a clear picture of population genetic structure and gene flow.

Stratified sampling was used to cover the range of spatial distribution within populations. A tape measure was placed parallel to the long axis of the population and sampling was done at more or less regular intervals along the tape in a zig-zag

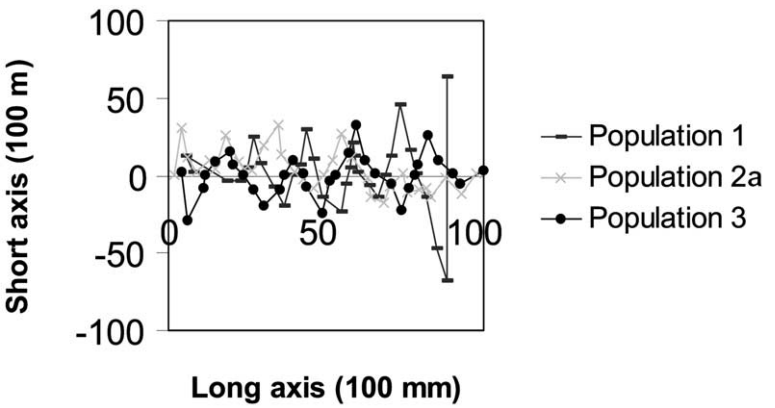

Fig. 2. Stratified sample distribution in three populations of *Barleria greenii*.

pattern across the full width of the population (Fig. 2) and sampled individuals were marked with tags in case there is a need for further investigations.

Young leaves were collected from growing shoots, placed in cryotubes and immediately submerged in liquid nitrogen (−196 °C). Leaf tissue extracts were prepared and analysed by starch gel electrophoresis using the extraction buffers, standard electrophoretic procedures, methods of interpretation of gel banding patterns and locus nomenclature followed by Van der Bank et al. (1995). Locus abbreviation, buffer systems used and enzyme commission numbers are given in Table 2.

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

Enzyme	Locus	E.C. No.	Buffer	pH
Asparatate aminotransferase	AAT-1,-2*[a]	2.6.1.1	LiOH[b]	8.1
Esterase	EST	3.1.1	MF[c]	8.6
Glucose-6-phosphate isomerase	GPI-1*,-2	5.3.1.9	MF	8.6
Isocitrate dehydrogenase	IDH*	1.1.1.42	HC[d]	6.5
Malate dehydrogenase	MDH-1*,-2*	1.1.1.37	MF	8.6
Malic enzyme	ME*	1.1.1.38	MF	8.6
Peptidase: Substrate		3.4-,-		
Leucine aminopeptidase	PEP-E		LiOH	8.1
Leucyl-tyrosine	PEP-S1*,-S2*		LiOH	8.1
Phenylalanyl-proline	PEP-D*		LiOH	8.1
6-Phosphogluconate dehydrogenase	PGD	1.1.1.44	HC	6.5
Phosphoglucomutase	PGM-1,-2	5.4.2.2	MF	8.6

[a] *=monomorphic loci
[b] LIOH: A discontinuous buffer (electrode pH 8.1; gel pH 8.3) system (Kephart, 1990)
[c] MF: A continuous buffer (pH 8.6) system (Market and Faulhaber, 1965)
[d] HC: A discontinuous buffer (electrode pH 6.5; gel pH 6.5) system (Kephart, 1990)

The BIOSYS-2 computer program (Swofford et al., 1997) was used to calculate average heterozygosity (*H*), coefficients of heterozygote deficiency (*d*), Chi-square (χ^2) values to test for deviations of allele classes from the Hardy–Weinberg proportions, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), Wright's fixation (*F*) indices and genetic distances (*D*) between all three populations. Levene's (1949) correction for small sample sizes was employed in the χ^2 analyses.

3. Results and discussion

3.1. Genetic variation

Sixteen enzyme-coding loci provided interpretable results in all populations analysed, of which seven (43.8%) displayed polymorphism. Nine of the 16 loci (56.2%) displayed monomorphic gel banding patterns (Table 2). This could be attributable to genetic bottlenecks associated with dispersal and subsequent establishment, or gradual isolation of larger populations into smaller ones as a result of climatic changes or increasing fire frequencies (Van der Bank et al., 1996). Staining of some enzymes, which provided interpretable results in a previous study for the genus *Barleria* did not give any results in this study. These enzymes included acid phosphotase, menadione reductase, peroxidase and superoxide dismutase (Van der Bank et al., 1999). Other enzymes, asparatate aminotranferase, esterase, malate dehydrogenase, malic enzyme and peptidases provided interpretable results but with buffers different from the previous study. However, HC 6.5 buffer provided best results in terms of band resolution for this study and the previous study of other species of *Barleria*.

Deviations of genotypic distributions from Hardy–Weinberg expectations occurred at two loci (GPI-2 and PEP-E) in Population 1, three loci in Population 2a (GPI-2, PEP-E and PGD) and five loci in Population 3 (AAT-1, GPI-2, PEP-E, PGD and PGM-1) (Table 3). This is not unusual since ideal Hardy–Weinberg populations seldom occur in nature owing to various factors which can shift the equilibrium and disrupt the stability and rare alleles of a population, giving rise to change in the genetic structure. Furthermore, significant deviations of allele classes may occur due to crossing and linking, inbreeding, sampling error, population bottlenecks and random genetic drift (Van der Bank et al., 1997). The genotypes at one locus (EST) in Population 1 and two loci (EST and PGM-2) in Populations 2a and 3 closely approximated the Hardy–Weinberg expectations (Table 3). Therefore, non-random mating and gene flow can be excluded in the present study since these processes generally affect all loci (Ayala, 1982). Deficiencies of heterozygotes occurred at three polymorphic loci (EST, GPI-2 and PEP-E) in Population 1, at GPI-2, PEP-E and PGD in Population 2a and at AAT-1, GPI-2, PEP-E, PGD and PGM-1 in Population 3. An excess (d=0.050) of heterozygotes only occurred at EST in Population 3 and d=zero was obtained at EST and PGM-2 in Population 2a and at the latter locus in Population 3. Monoallelic states were observed at AAT-1 and PGM-1 in Population 1 and 2a, and at PGD and PGM-2 in Population 1. Gradual (clinal) decreased frequencies of the most common alleles were obtained at PGD and PGM-1 from Population 1 to 3.

Table 3
Relative allele frequencies, coefficients of heterozygosity deficiency (negative *d* values) or excess values for polymorphic loci are listed after each enzyme

Locus	Relative mobility and heterozgosity deficiency (*d*)	Population		
		1	2a	3
AAT-1	120	1.000	1.000	0.967
	100			0.033
	(d)			(−1.000)
EST	−100	0.333*[a]	0.050*	0.091*
	95	0.667	0.950	0.909
	(d)	(−0.528)	(0.000)	(0.050)
GPI-2	70	0.063	0.113	0.083
	75	0.938	0.887	0.917
	(d)	(−1.000)	(−0.842)	(−0.785)
PEP-E	100	0.387	0.129	0.196
	90	0.613	0.839	0.768
	87		0.016	
	85		0.016	0.036
	(d)	(−0.599)	(−0.659)	(−0.527)
PGD	100		0.083	0.143
	95	1.000	0.917	0.857
	(d)		(−1.000)	(−1.000)
PGM-1	110	1.000	1.000	0.857
	108			0.143
	(d)			(−1.000)
PGM-2	100	1.000	0.958*	0.929*
	97		0.042	0.071
	(d)		(0.000)	(0.000)

[a] *Polymorphic loci where allele classes conformed to expected Hardy–Weinberg proportions

The *A* values were 1.19 (±0.19) in Population 1, 1.44 (±0.20) in Population 2a and 1.50 (±0.16) in Population 3. The *H* values were 0.065 (±0.039), 0.050 (±0.022) and 0.086 (±0.030) and the *P* values were 18.75, 31.25 and 43.75% for Populations 1, 2a and 3 respectively (Table 4). Values of *A* and *P* increased progressively from

Table 4
Mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*) using the 0.95 criterion and average heterozygosity

Population	*A*	*P* (%)	*H*
1	1.19 (±0.19)	18.75	0.065 (±0.039)
2a	1.44 (±0.20)	31.25	0.050 (±0.022)
3	1.50 (±0.16)	43.75	0.086 (±0.030)
Average for endemics[a]	1.42	28.4	0.069

[a] Hamrick and Godt (1989)

the east (Population 1) to the west (Population 3) thus indicating clinal variation whereby genetic diversity increases with geographical distance. This suggests that *B. greenii* has undergone gradual genetic divergence following isolation of populations. In a review of the allozyme literature, Hamrick and Godt (1989) indicated that the average genetic diversity statistics for endemic populations is 28.4% for the *P* value, 1.42 for *A* and 0.069 for the *H* value. They also found that endemic and rare taxa often contain significantly less genetic variability and in several cases, have higher levels of self-pollination and inbreeding. Schoen and Brown (1991) and Brown and Schoen (1992), however, argued that the uncritical application of such generalizations could lead to serious errors, particularly with selfing species. They demonstrated that genetic diversity within populations varies more among populations of selfing species than among populations of predominantly outcrossing species. Some populations of self-pollinating taxa are polymorphic at several loci and may have high levels of genetic diversity, whereas other populations are largely monomorphic. Since other *Barleria* species have not been analysed, it is not known whether *B. greenii* has lower levels of genetic diversity than more widespread congeners, but in any event the level of genetic diversity measured for *B. greenii* is somewhat higher than the means for endemic species except for the values in Population 1 of 0.069.

Individual number 3.16 from Population 3 had a rare PEP-E*85 allele and individual 2.5 from Population 2a had two rare alleles (PEP-P*85 and PEP-P*87) (Table 3). Rare alleles are alleles which are found at low frequencies within a population. These alleles are thought to have been selected against; there is a possibility that their fitness is low. Rare alleles are particularly sensitive to gene flow because it is unlikely that they will be carried by dispersing gametes or individuals unless dispersal is frequent. It might also be possible that these alleles were missed in Population 1 when sampling and it would be interesting to see if they have morphological characters associated with these genotypes.

3.2. Genetic differentiation

Nei's (1978) unbiassed genetic distance (*D*) adapted for small sample size was 0.008 between Populations 1 and 2, 0.006 between Populations 1 and 3 and zero between Populations 2a and 3. There is a distinct association between the genetic and geographical distances with the latter two populations being the closest to each other and do not show any significant ($\rho<0.05$) differences genetically (see the contingency χ^2 values below) (Table 5). The relatively low *D* values between Populations 1 and 2a, and Populations 1 and 3 suggest little genetic differentiation since these populations shared a common ancestor. Thorpe (1982) and Thorpe and Solé-Cava (1994) estimated a mean *D* value of 0.03 for conspecific populations and Van der Bank et al. (2001) estimated a *D* value necessary to distinguish between plant populations to be 0.05. The *D* values for this study are much less. The cophenetic correlation value for the Nei's (1978) unbiassed genetic distance is 96.2, 99.6% for Nei's trees (1972) genetic distance and 100% for the chord distance coefficient of Cavalli-Sforza and Edwards (1967) (Fig. 3). The bootstrap value (1000 replicates)

Table 5
Nei's (1978) genetic distance (*D*) geographical distance, pair-wise contingency Chi-square, summary of *F*-statistics values at all polymorphic loci and the effective number of individuals exchanged in each generation, N_{em}

Populations	*D*	Distance	Chi-square	F_{IS}	F_{IT}	F_{ST}	N_{em}
1 and 2a	0.008	3.235	0.00401*[a]	0.605	0.635	0.076	1.946
1 and 3	0.006	4.873	0.00893*	0.605	0.626	0.054	1.351
2a and 3	0	1.914	0.42486	0.611	0.617	0.015	7.296

[a] *significant $\rho<0.05$

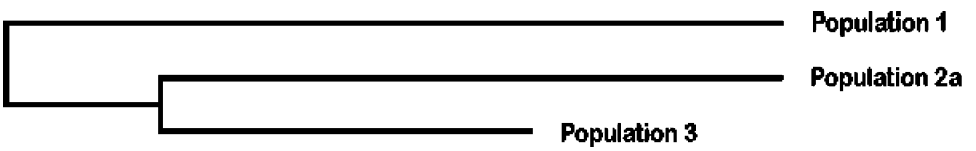

Fig. 3. Genetic differentiation in three populations of *Barleria greenii* using Wagner tree produced by Cavalli-Sforza and Edwards' (1967) chord distance values by rooting at the midpoint of the longest path.

at all nodes is 100%. Population 1 is more differentiated from the other two populations that grouped together. This is evident from the long branch length in the Wagner tree (Fig. 3).

Wright's (1965, 1978) pair-wise, average weighted measure of differentiation (F_{ST}) of individuals between Populations 1 and 2a (0.076) and Populations 1 and 3 (0.054) is an indication of moderate genetic differentiation (Table 5). The F_{ST} value of 0.015 between Populations 2a and 3 is indicative of little genetic differentiation i.e. 94.6, 92.4 and 98.5% of the differentiation was within the above mentioned populations compared to 1.5–7.6% between population differentiation.

The pair-wise contingency χ^2 analysis at all loci indicated significant (ρ=0.009) differences in mean allelic distribution between Populations 1 and 3. The locus that contributed most to population differences was PEP-E with a ρ value of 0.033. Significant difference was also observed between Populations 1 and 2a with an overall ρ value of 0.004 with PEP-E (0.007) and EST (0.024) contributing most to population differentiation. No significant difference was observed between totals for Populations 2a and 3 (ρ=0.425). However, most loci between Populations 1 and 2a (GPI-2, PGD and PGM-2), between Populations 1 and 3 (AAT, GPI-2, PGD, PGM-1 and PGM-2) and all loci between Populations 2a and 3 did not show significant differences, suggesting that there has not been enough time since their separation to reflect divergence because they still have more common alleles. Mean *F* values of populations relative to the total population; F_{IS}, was 0.605, 0.605 and 0.611 between the above-mentioned populations respectively (Table 5). The F_{IT} value indicates the amount of inbreeding in the population due to population sub-division. It was found to be high 0.635 between Populations 1 and 2a, 0.626 between Populations 1 and 3 and 0.617 between Populations 2a and 3 (Table 5). The high F_{IT} and F_{IS} values between

all populations is indicative of high inbreeding rates hence showing barriers to gene flow.

Our sample size could be considered as sufficient to detect the differentiation at diploid loci among populations (using F_{ST}; see Nei (1973) with a type I error at 5%. The average F_{ST} value among all three pairs of populations is 0.048, to detect a F_{ST} value of 0.05 just ten diploids per locality may be adequate (Baverstock and Moritz, 1996) using the formula of where: $2n=1/F_{ST}$ (Chakraborty and Leimar, 1987; Slatkin and Barton, 1989). In addition, the populations studied can be regarded as a single unit (see discussions below); therefore, the ranges might present a true reflection of the amount of genetic variation and differentiation for the total of 96 individuals of *B. greenii* studied. Furthermore, no statistically significant difference between heterozygosity values was obtained when fewer (25 compared to 50) individuals were analysed in *Virgilia oroboides* (Van der Bank et al., 1996).

Seeds in *B. greenii* are dispersed over short distances. The effect of this is that gene flow is likely to be restricted because pollinators visit plants that are near to each other. Observations suggest that seed parasitism is very high in *B. greenii* i.e. in instances where there might have been successful pollination and seed production, survival of the seed into the next generation is prohibited by seed parasites. Population 1 has the lowest level of genetic variation, depicted by the *A* and *P* values, and is much more differentiated as compared to the other populations (Fig. 3). This may be a result of isolation by distance (Fig. 1). Short seed dispersal distance, seed parasitism, and local extinction, possibly due to fires and isolation by distance are considered to be the most likely explanations for the low genetic divergence encountered in the studied populations.

It has been observed that gene flow estimates from the effective number of individuals exchanged in each generation (N_{em}) do not allow conclusions to be drawn about whether a species or group of species is endangered (Packer and Owen, 2001). This is because different population processes associated with endangerment may lead to both high and low estimates of N_{em}. Low levels of N_{em} may indicate insufficient cohesion among sample populations to prevent genetic drift from leading to speciation, or even the likelihood that speciation has already occurred. Low estimates of gene flow may also indicate dangerous levels of population isolation such that extirpation of any of them is likely to be permanent. Conversely, high levels of N_{em} may result from factors other than high contemporary rates of gene flow. For example the results of historically high levels of gene flow that are no longer in operation. Alternatively, they may indicate ephemeral metapopulation structure with high extinction rates in which all but one, or a few, subpopulations have recently been extirpated, with subsequent relocalization from one, or a few, subpopulations (Packer and Owen, 2001). High estimates of N_{em} may also indicate frequent local extinction and relocalization especially when combined with low heterozygosity values (Waller et al., 1987). Slatkin (1985) indicated that populations that have smaller genetic distances between them are assumed to have higher levels of gene flow than populations that have larger genetic distances. This is evident from the N_{em} values of the present study; it was 1.947 between Populations 1 and 2a, 1.351 between Populations 1 and 3 and 7.296 between Populations 2a and 3 using the relationship between F_{ST}

and N_{em} (Takahata, 1982). This latter high value suggests that there is substantial gene flow between the populations to counter the effects of genetic drift. The geographical distance, *D*, and F_{ST} are the lowest between Populations 2a and 3 whereas the N_{em} value is the highest. The opposite is true between Population 1 and the other two populations. On this basis, we predict that pollinators will fly long enough distance to move from population to population and that the entire group studied may consist of a single random mating unit.

The sites where *B. greenii* occurs are well conserved at present, falling either within the boundaries of the Lowlands East Conservancy or within the Weenen Nature Reserve. However, because of its highly restricted distribution and specific habitat requirements, it is regarded as rare and its conservation status should continue to be carefully monitored (Balkwill et al., 1990). There is also the proposed Gongolo Wildlife Reserve, which will be constructed in 2-years time. Associated with the establishment of the reserve will be a burning plan, roads, lodges and other developments. It is very important that these developments should not be in areas of *B. greenii* i.e. the road runs through Population 3 and because where short distance dispersal it could fragment the population. Therefore establishing anything in the area between the populations should be avoided. The Mielietuin Dam (Fig. 1) is also going to be built but not in any area between the populations so will not have any impact on *B. greenii*.

3.3. Conclusions

The probability of seeds being dispersed from one population to another is extremely low because of short distance dispersal and highly reduced numbers of seeds due to seed parasitism. The combination of these factors with isolation by distance reduces gene flow. Isolation by distance, whether by geographic, ecological or behavioural factors, will prevent the influx of new alleles and the end result can be a highly inbred population with low viability (Schonewald-Cox et al., 1983). The low genetic variability in *B. greenii* may also be a result of frequent and infrequent local extinction, possibly due to fires; therefore the fire regime should favour the establishment of seedlings and resprouting of the individual plants of *B. greenii*. Management plans should also consider the effects of parasites and how to conserve diversity in a dynamically evolving system. New roads shouldn't be constructed where populations will be fragmented and consideration should be given to re-routing the road through Population 3. If new populations were to be established in a suitable habitat in which populations do not occur, it might conserve more infraspecific diversity if the plants with rare alleles were used for these purposes. It is important that every single population of *B. greenii* should be conserved. Populations 2a and 3 maintain the most genotypic diversity and Population 1 has the lowest amount of genetic variability but deserves to be conserved to ensure survival and evolutionary capacity of the taxon by preserving the dynamic capacity of the species to evolve.

It can be predicted from this study that gene flow promotes concentration of the rare alleles in the central populations (e.g. Populations 2a and 3; Fig. 1; i.e. it consists of a continuous population with individuals within it exchanging genes with geo-

graphically proximate individuals—the isolation-by-distance model; Baverstock and Moritz (1996). It would be interesting to investigate how differentiated these populations are from other populations, particularly Populations 6–8. Therefore, further studies should focus on determining population genetic structure in as many as possible of the other populations in order to provide insights on how geographical distance between populations indicates levels of differentiation and of gene flow among them.

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