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Allozyme variation in *Barleria saxatilis* (Acanthaceae) is lower than in two congeneric endemics

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Genetic variability in *Barleria argillicola* and *B. greenii*, two sympatric and endemic species restricted to Estcourt in KwaZulu-Natal, was compared with *B. saxatilis*, a closely related widespread species inhabiting dry hot areas in KwaZulu-Natal, Mpumalanga, Limpopo and Swaziland bushveld. Three populations from each species were sampled for electrophoretically detectable diversity. In contrast to expectations based on similar surveys in other plants, the widespread species showed reduced within-population gene diversity with respect to its endemic congeners. The relationship between the observed levels of allozyme diversity and the mating system in each of the three *Barleria* species is discussed.

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 showing a high degree of regional endemism. Endemism in many cases seems to be attributable to edaphic factors. Approximately 45 of the 70 species in southern Africa are endemic, some with highly restricted distributions spanning only one- or two-quarter degree squares (Balkwill and Balkwill 1998). Two such endemics are *B. greenii* M-J. & K. Balkwill and *B. argillicola* Oberm. Both of these species are listed as vulnerable in the Red Data book (Hilton-Taylor 1996). *Barleria saxatilis* Oberm. is a related and widespread (Northern Province, Mpumalanga, Swaziland and KwaZulu-Natal) congener which has been included in this study to determine if there are substantial differences between genetic factors associated with rarity and endeminism and those affecting populations of more common species. *Barleria argillicola* is clonal, constantly sparse and geographically restricted in specific habitat at Estcourt (South Africa). *Barleria greenii* on the other hand is locally abundant in specific habitats but also geographically restricted to Estcourt. Their widespread congener, *B. saxatilis*, is locally abundant over a large range in several habitats hence a good example of a species that expands its distribution by the colonisation of new habitats in several regions.

The selection of *B. saxatilis* as the most closely related widespread species was based on species found in South Africa in the Section *Barleria*. The section has been subdivided into a number of informal groups based on variation in particular characters (Balkwill and Balkwill 1998). Group 1 consists of 37 species including species with widespread and restricted geographical distributions. Twelve of these species are found in South Africa including *B. argillicola*, *B. greenii* and *B. saxatilis*. *Barleria saxatilis* was chosen as the most widespread congener because it is found in the same region as the endemics and so the differences in population structure are more likely to be factors influencing rarity, rather than being due to differences in environmental conditions.

Little is known about the genetic structure of the species in the genus *Barleria*, the only published studies with allozyme data have differentiated between species (Van der Bank *et al.* 2000) or determined population genetic structure of *B. greenii* (Makholela *et al.* 2003) and *B. argillicola* (Makholela *et al*. in press). In addition, no information is available on comparison of population genetic structure between rare and widespread species. This study was therefore initiated to determine the population genetic structure of the widespread *B. saxatilis* so that it could be compared with that of the rare and locally endemic taxa, *B. argillicola* and *B. greenii*, of the same genus in order to determine genetic factors associated with rarity. The levels of genetic diversity observed are compared with the type of mating system in these species. This information might facilitate management of these rare species. The three species

appear similar in life history traits: long-lived perennial herbs/shrubs with temperate-tropical regional distribution, zoophilous pollination, outcrossing breeding system, sexual reproduction and explosive short distance seed dispersal (K Balkwill pers. obs.).

Materials and Methods

Leaf samples of *B. greenii*, *B. argillicola* and *B. saxatilis* were collected from nine natural populations representing the three taxa studied in May 2001. From these natural populations 32 individuals per population were sampled. *Barleria greenii* occurs in eight localities near Estcourt. Of these, only three sites were visited to collect samples for isozyme analysis (Table 1). The first population of *B. greenii* was collected from the type locality on the farm Van der Merwe's Kraal and the rest from the farm Selbourne (Table 1). The distance between populations was calculated using the latitude and longitude (Table 1); it is 4.87km between Populations 1 and 3, 3.24km between Populations 1 and 2, and 1.91km between Populations 2 and 3. *Barleria argillicola* was collected from three localities on the farm Van der Merwe's Kraal, the only three known sites where at least 30 individuals occurred (Table 1). A fourth site was known at the time, but only 15 individuals could be found. Since the study, another few localities have been found. The distance between Populations 1 and 2 is 3.20km, 4.80km between Populations 1 and 3 and 1.70km between Populations 2 and 3. *Barleria saxitilis* is known from many localities but samples were collected from only three (Table 1). The distance between Populations 1 and 2 is 79.55km, 85.74km between Populations 1 and 3 and 12.39km between Populations 2 and 3.

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 intervals along the tape within the population. Individuals were sampled in a zig-zag pattern across the full width of the population and were then marked with plant 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 buffer, standard electrophoretic procedures, method of interpretation of gel banding patterns and locus nomenclature followed by Van der Bank *et al*. (1995). Locus abbreviation, buffer systems to be used and enzyme commission numbers are given in Table 2.

The BIOSYS-2 computer program (Swofford *et al.* 1997) was used to calculate average heterozygosity (H), Chi-square (χ^2) values, mean number of alleles per locus (A), percentage of polymorphic loci (P) and Nei's (1972) genetic distances (D) between all three populations. Levene's (1949) correction for small sample sizes was employed in χ^2 analyses.

The levels of genetic diversity were compared with the mating system by field experiments in which *B. argillicola* and *B. greenii* were compared for fruit set in the absence and presence of pollinators in order to determine capacity

Table 1: Sites from which samples were collected for the study

Species	Population	Locality	Latitude	Longitude	Altitude (m)
B. greenii	1	Estcourt on the farm Van Merwe's Kraal 972	28°56.58'S	29°58.16'E	1 231
	2	Estcourt on the farm Selbourne	28°55.94'S	30°00.74'E	1 211
	3	Estcourt on the farm Selbourne	28°56.17'S	29°59.66'E	1 190
B. argillicola	1	Estcourt on the farm Van Merwe's Kraal 972	28°59.43'S	29°56.91'E	1 119
	2	Estcourt on the farm Van Merwe's Kraal 972	28°57.79'S	29°58.40'E	1 135
	3	Estcourt on the farm Van Merwe's Kraal 972	28°57.51'S	29°58.94'E	1 203
B. saxatilis	1	Letaba (The Downs) on the farm Gemini 62KT	24°06.35'S	30°07.31'E	808
	2	Letaba on Chester farm 235KT	24°27.59'S	30°48.17'E	720
	3	Along the Blyde River Canyon Nature Trail	24°33.99'S	30°47.49'E	954

Table 2: Enzyme locus abbreviations, enzyme commission numbers (EC No.), buffers and the pH at which they were used

Enzyme	Locus	EC No.	Buffer	pH
Asparatate aminotransferase	Aat-1*, -2*	2.6.1.1	LiOH[a]	8.1
Glucose-6-phosphate Isomerase	Gpi-1, -2*	5.3.1.9	MF[b]	8.6
Isocitrate dehydrogenase	Idh*	1.1.1.42	HC[c]	6.5
Malate dehydrogenase	Mdh-1*, -2*	1.1.1.37	MF	8.6
Malic enxyme	Me*	1.1.1.38	MF	8.6
Peptidases: Substrate Leucyl-tyrosine	Pep-S1, -S2*	3.4-,-	LiOH	8.1
Phosphogluconate dehydrogenase	Pgd*	1.1.1.44	HC	6.5
Phosphoglucomutase	Pgm-1*, -2*	5.4.2.2	MF	8.6

* monoallelic loci

[a] LiOH: A discontinuous buffer (electrode pH 8.1, gel pH 8.3) system (Kephart 1990)

[b] MF: A continuous buffer (pH 8.6) system (Market and Faulhaber 1965)

[c] HC: A discontinuous buffer (electrode pH 6.5, gel pH 6.5) system (Kephart 1990)

for autonomous self pollination. Plants were selected and covered with shade netting in order to exclude pollinators before pollination commenced. Equivalent numbers of plants for each species were selected and treated as controls in *B. greenii* and *B. argillicola*. One-way ANOVA was used to compare fruit set in the absence and presence of pollinators. For *B. saxatilis* observations on fruit set and floral polymorphism were made in the phytotron. In addition, pollen grains were counted from young flower buds in each species by cutting the anthers into small pieces. Each of these pieces was placed on a slide and 70% ethanol was added to allow the pollen grains to be spaced throughout the slide. Counting all the pollen grains using a microscope then followed. The total number of ovules (O) counted from immature ovaries were divided by the number of pollen (P) grains to get the P:O ratio. Lastly, morphological characters indicative of mating breeding systems were used to obtain the outcrossing index (Cruden 1977). The characters included diameter of the flower, temporal separation of anther dehiscence and stigma receptivity and spatial positioning of the stigma and anthers.

Results

Of the 22 loci scored in a previous study (Van der Bank *et al.* 2000), 13 enzyme-coding loci provided interpretable results in all populations in the present study, of which two (15.4%) displayed polymorphism. Eleven (84.6%) displayed monoallelic gel banding patterns. All individuals were homozygous for the same allele at all loci in Populations 1 and 2. In contrast, the two loci, Gpi-1 and Pep-S1, showed pronounced polymorphism, each with two electrophoretic variants with relative mobilities of 80 and 100 respectively in Population 3. Low levels of heterozygosity were observed in Population 3 (0.017 ± 0.13). The other populations (1 and 2) had an H value of zero. In addition Population 3 exhibited a higher A value 1.2 (±0.1) compared to the other populations with an A value of 1.0 (±0.0). Deviations of genotypes from Hardy-Weinberg equilibrium were observed at the Gpi-1 (allele frequency = 0.926 for Gpi*100 and 0.074 for Gpi*80) locus but not at the Pep-S1 locus (allele frequency = 0.964 and 0.036 respectively).

All measures of variability calculated from allozyme data demonstrate the widespread species, *B. saxatilis*, maintains lower levels of genetic diversity than the two endemics despite the greater geographical distance between populations of the widespread species. At the species level, the endemics (*B. argillicola* and *B. greenii*) had similar genetic diversity for A and P (1.3 ± 0.1% and 33% respectively). These values were higher than their widespread congener, with A (1.2 ± 0.1) and P (16.7%). The H values averaged 0.021 (±0.1) in *B. argillicola*, 0.036 (±0.017) in *B. greenii* and 0.006 (±0.004) in the widespread species (*B. saxatilis*). The enzyme loci used in the previous studies of *Barleria greenii* (Makholela *et al*. 2003) and *B. argillicola* (Makholela *et al.* in press) also provided interpretable results for *B. saxatilis* except for asparatate aminotransferase, esterase and leucine aminopeptidase.

The mean inter-specific D values between *B. argillicola* and *B. greenii* were 0.374, 0.387 between *B. greenii* and *B. saxatilis* and 0.287 between *B. argillicola and B. saxatilis*. Intra-specific D values for all populations of *B. argillicola* and *B. saxatilis* ranged from 0 to 0.001, with a mean of 0.0006; for *B. greenii*, D values ranged from 0.002 to 0.01, with a mean of 0.007. With such a small number of populations it was impossible to test genetic and geographical distances for statistical significance.

There was no significant difference (P = 0.06) between fruit set in bagged and unbagged plants in *B. greenii*. In general fruit set was very low in both bagged (0.072 ± 0.018) and unbagged (0.085 ± 0.020) flowers (mean ± SE) (n = 50). The same was observed in *B. argillicola* with no significant difference, P = 0.322, between fruit set in the absence (n = 23) and presence (n = 23) of pollinators. Fruit set was generally low because out of the 46 individuals sampled, only seven individuals set fruit in the absence of pollinators and 11 when pollinators were not excluded. In *B. saxatilis*, cleistogamous and chasmogamous flowers were observed. Fruit set was also observed in both cleistogamous and chasmogamous flowers.

The type of breeding system obtained from P:O was facultative autogamy in all the three *Barleria* species. Cruden's (1977) outcrossing index indicated *B. greenii* to be partially self-compatible, outcrossing and with a demand for pollinators. The same was also observed in *B. argillicola* and *B. saxatilis*, but it was also observed that *B. argillicola* can be self-compatible with some demand for pollinators whereas *B. saxatilis* can also be cleistogamous.

Discussion

Reproductive assurance, maintained through a selfing mechanism and short distance seed dispersal, might be important in the recruitment and survival of all three species of *Barleria*. There is recruitment through clonal growth (Figure 1) and seedlings (Figure 2) in *B. argillicola*. The species is also self-compatible as demonstrated by P:O, outcrossing index and fruit set when pollinators are excluded. Flowers are produced that have the stigma above the anthers (Figure 3a), and both the stigma and anthers at the same level, thus promoting autonomous self pollination since the species is self-compatible (Figure 3b). In the former case, the anthers will make contact with the stigma as the corolla is shed (Figure 4). However, in some flowers, the stigmas curve away from the anthers and are thus unlikely to make contact with the anthers. *Barleria greenii* is also self-compatible and can set seeds when pollinators are excluded, which suggests the existence of a selfing mechanism (unpublished data). Their widespread congener, *B. saxatilis*, has both cleistogamous (Figure 5) and chasmogamous (Figure 6) flowers, both of which can set seeds in the absence of pollinators. Acanthaceae display mechanical seed dispersal after explosive fruit dehiscence (Bremekamp 1965). This leads to short distance seed dispersal and limits the number of immigrants to new suitable habitats. The cleistogamy displayed by *B. saxatilis* would guarantee seed set in a single individual but would reduce the levels of genetic diversity in populations, which could then be founded by single individuals.

Flower size and corolla tube length were measured due to

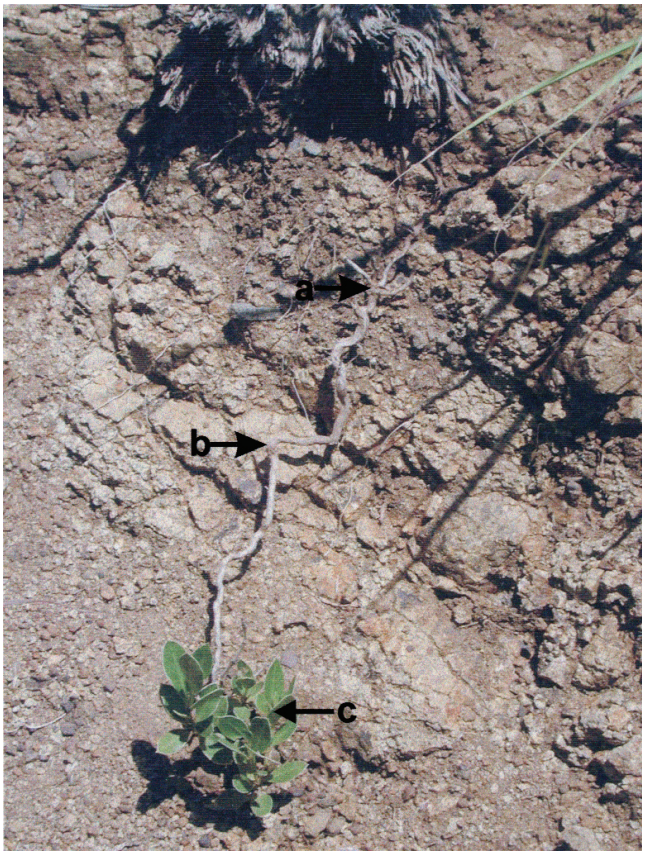

Figure 1: Clonal growth in *B. argillicola*; previous crown of plant (a), root from original crown (b), stems that have sprouted from new crown formed on root (c)

Figure 2: Seedling amongst plants in Population 3 of *B. argillicola*; cotyledon (a)

Figure 3: *Barleria argillicola* flowers in which the anthers and stigma are at different levels (a) and anthers and stigma at the same level (b)

Figure 4: Shed corolla in *B. argillicola* promoting contact between anthers and stigma

Figure 5: *Barleria saxatilis* cleistogamous flower, in which self-fertilisation occurs in the unopened flower, in the greenhouse

Figure 6: *Barleria saxatilis* chasmogamous flowers below which fruits are developing despite pollinators being excluded from the phytotron

the occurrence of two different floral displays in *B. argillicola* to determine if there will be any differences in flowers in which both anthers and stigma are at the same level, and those in which the stigmas are above the anthers using one way ANOVA. Results revealed significant differences (P = 0.016) in flower size (n = 38) with more variance in flowers in which stigmas are above anthers (21.728) compared to 4.589 of flowers in which both anthers and stigmas are at the same level. However, there was no significant difference (P = 0.775) in corolla tube length (n = 38). Thus flower size but not corolla tube length could be used to differentiate between different floral displays in *B. argillicola*, with flowers in which both the anthers and stigmas are at the same level having smaller flower size (than the ones in which anthers and stigmas are spatially separated).

In a review of 38 studies that directly compare the ecological and demographic attributes of a rare species with those of a closely related more common species, little consistency and almost no comparability among studies was found (Bevill and Louda 1999). For example, of 71 response variables compared between rare species and their common relatives, 60 (85%) were reported in less than four comparisons. Only seven variables (10%) were reported in more than four of the 38 comparisons: pollination biology, seed set, seed size, seed bank, competitive ability, phenotypic variation and genetic variation. These comparisons suggest that a set of variables should be collected, and should include parameters critical in determining and comparing changes in population size and persistence, specially birth and death rates (Bevill and Louda 1999). Fielder (1987) indicated that differences in density and distribution might be intrinsic and species-specific. Alternatively, density and distribution may be limited differently for each type of rarity (Rabinowitz and Rapp 1981) or differences may represent variation in the intensity of limiting factors rather than the influence of different mechanisms among categories of abundance (Gaston 1994). Schemske *et al*. (1994) further indicated that the persistence of locally rare and geographically restricted species likely depends more on demographic traits and population dynamics than on genetic structure. Therefore, the factors used in this study (genetic variation and mating systems) could not be used as good indicators of factors associated with rarity and endeminism and those affecting population genetics of more common species since all three species studied generally have lower levels of genetic diversity, mixed mating systems and existence of a selfing mechanism. However, the results from allozyme data could clearly be explained using data derived from observations of characters relating to mating systems.

Conclusion

We could not establish what it is that makes *B. argillicola* and *B. greenii* rare and endemic using allozyme data and mating systems, but we were able to determine that cleistogamy is responsible for the widespread distribution of *B. saxatilis* whereas reproductive assurance and short distance seed dispersal are responsible for recruitment and establishment of all three species of *Barleria*.

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References

Balkwill MJ, Balkwill K (1998) A preliminary analysis of distribution patterns in a large pantropical genus, *Barleria* L. (Acanthaceae). Journal of Biogeography **25**: 95–110

Bevill RL, Louda SM (1999) Comparisons of related rare and common species in the study of plant rarity. Conservation Biology **13**: 493–498

Bremekamp CEB (1965) Delimitation and subdivision of the Acanthaceae. Bulletin of the Botanical Survey of India **7**: 21–30

Cruden RW (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution **31**: 32–46

Fielder PL (1987) Life history and population dynamics or rare and common mariposa lilies (Calochortus Pursh: Liliaceae). Journal of Ecology **75**: 977–995

Gaston KG (1994) Rarity. Chapman and Hall, London

Hilton-Taylor C (1996) Red Data List of Southern African Plants. National Botanical Institute, Pretoria

Kephart SR (1990) Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. American Journal of Botany **77**: 693–712

Levene H (1949) On a matching problem arising in genetics. The Annals of Mathematical Statistics **20**: 91–94

Makholela TM, Van der Bank FH, Balkwill K (2003) A preliminary study of allozyme variation in three rare and restricted endemic *Barleria greenii* (Acanthaceae) populations. Biochemical Systematics and Ecology **31**: 141–154

Makholela TM, Van der Bank FH, Balkwill K (submitted) Red data assessment, allozyme variation, pollination biology and management strategies in rare and restricted South African endemic, *Barleria argillicola* (Acanthaceae) populations. Taxon

Market CL, Faulhaber I (1965) Lactate dehydrogenase, isozyme patterns of fish. Journal of experimental Zoology **159**: 319–332

Nei M (1972) Genetic distance between populations. American Naturalist **106**: 283–292

Rabinowitz D, Rapp JK (1981) Dispersal abilities of seven sparse and common grasses from a Missouri prairie. American Journal of Botany **68**: 612–624

Schemske DW, Husband BC, Ruckelshaus MH, Goodwillie C, Parker IM, Bishop JG (1994) Evaluating approaches to the conservation of rare and endangered plants. Ecology **75**: 584–606

Swofford DL, Selander RB, Black WL (1997) BIOSYS-2. A Computer Program for the Analysis of Allelic Variation in Genetics. Department of Microbiology, Colorado State University

Van der Bank FH, Van der Bank M, Balkwill M-J, Balkwill K (2000) Electrophoretic evidence for an undescribed species of *Barleria* L. South African Journal of Botany **66**: 22–27

Van der Bank FH, Van Wyk B-E, Van der Bank M (1995) Genetic variation in two economically important *Aloe* taxa (Aloaceae). Biochemical Systematics and Ecology **23**: 251–256

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