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Low impact of fragmentation on genetic variation within and between remnant populations of the typical renosterveld species *Nemesia barbata* in South Africa

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ABSTRACT

Renosterveld is a Mediterranean-type shrubland in the south-western Cape of South Africa. It is an exceptionally species-rich habitat and a local biodiversity hotspot. However, it has been strongly fragmented due to land use intensification during the last centuries. We analysed the impact of fragmentation on the genetic variation of a typical renosterveld species, the annual herb *Nemesia barbata*. For our investigation we selected populations of the species in 20 renosterveld fragments of different sizes in the Cape lowlands and determined genetic variation within and between populations using amplified fragment polymorphsims (AFLPs). We expected genetic pauperisation within small and isolated fragments and a lack of gene flow between these fragments.

We observed considerable genetic variation within but only a low level of variation between populations. Genetic variation within populations was not correlated with the size of the fragment or the distance to the nearest adjacent fragment. However, genetic variation between populations was positively correlated with geographic distance between fragments, indicating historical and/or actual gene flow.

Based upon our results, we conclude that habitat fragmentation does not yet influence the genetic variation of *N. barbata*. Historical and possibly actual gene flow, combined with buffering effects of the soil seed bank, appear to have minimized the negative impacts of habitat fragmentation on genetic variation of this renosterveld species.

1. Introduction

Habitat fragmentation leads to habitat loss, reduced habitat size (Luijten et al., 2000) and smaller population sizes (Bruna and Kress, 2002). Eventually, it may result in reduced gene flow (Ouborg et al., 2006), loss of genetic variation (Fahrig, 2001), inbreeding and drift (Young et al., 1996), and the decrease of short- and long-term population viability (Ellstrand and Elam, 1993).

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Both ecological and genetic processes can influence the genetic variation of species and populations, which underpins their short- (Huenneke et al., 1991) and long-term persistence (Lee et al., 2006). Genetic variation strongly depends on plant functional traits, e. g. longevity and woodiness (Hamrick and Godt, 1996; Nybom and Bartish, 2000). Habitat fragmentation induced reduction of population size can be responsible for the decrease of genetic variation (Oostermeijer et al., 2003). This relationship is mostly positive and stronger in self-incompatible than self-compatible plants (Leimu et al., 2006). Montalvo et al. (1997) report that small populations are more affected by genetic drift than larger ones. However, it is necessary to acknowledge population distance as a parameter in order to evaluate the spatial genetic structure (Ouborg et al., 2006). Both neutral (Young et al., 1993) and negative effects (Schmidt and Jensen, 2000) on genetic variation are reported.

Maintenance of genetic variation in fragmented populations is a major conservation goal in order to avoid inbreeding depression (Saccheri et al., 1998) and to ensure adaptation (Hedrick and Miller, 1992). Gene flow by means of pollen and diaspores ensures adaptation and evolution (Heywood, 1991), and habitat fragmentation can thus result in reduced gene flow and genetic variation (Young et al., 1996). While naturally fragmented plant populations are relatively prone to inbreeding depression (Huenneke et al., 1991), anthropogenic induced fragmentation can result in reduced genetic variation (Lande and Barrowclough, 1987).

South African renosterveld vegetation is a species-rich shrubland, found in a Mediterranean-type climate and on nutrient-rich soils (Rebelo et al., 2006). Renosterveld occupied large proportions of the south-western Cape lowlands (Kemper et al., 1999), but nowadays it is highly fragmented and endangered, as mainly agricultural land-transformation destroyed ninety-two percent of the former extent (von Hase et al., 2003).

We analysed the impact of fragmentation on the genetic variation of a typical renosterveld species, *Nemesia barbata*, using amplified fragment length polymorphisms (AFLPs). Specifically we asked, (i) whether genetic variation within populations decreases with the size of the fragment or the distance to adjacent fragments and (ii) whether genetic variation between fragments increases with the distance between them.

2. Material and methods

2.1. Species description

N. barbata (Thunb.) Benth. (Hemimerideae, Scrophulariaceae, Olmstead et al., 2001) occurs in low abundances on sandy flats and slopes in the western Cape Floristic Region (Goldblatt and Manning, 2000). The annual herb flowers from August to October, distinguished by white upper lip and blue lower lip and a short single spur. It shows a wide variation in flower size and colour. The plant grows up to 30 cm in height and has opposite, ovate and toothed leaves (Goldblatt and Manning, 2000). *N. barbata* has a mixed mating system and is self-compatible (Datson et al., 2006). It is insect-pollinated and adapted for pollination by hopliine beetles (Goldblatt and Manning, 2011). Seeds are small and dispersal via gravity or herbivores can be assumed.

2.2. Sampling procedure

We sampled plant material for molecular analyses in fragmented Swartland Shale Renosterveld and Granite Renosterveld (Rebelo et al., 2006). The study area spanned up to 40 km north and east of Cape Town in the Cape lowlands. Here, we selected 20 renosterveld fragments where *N. barbata* occurred (Table 1). Where available we collected fresh leaf material of 20 individuals for the analysis of genetic variation with AFLPs. Since it is nearly impossible to determine the number of individuals per renosterveld fragment in the field, we used habitat size as a surrogate for population size as demonstrated in previous studies.

2.3. DNA isolation and AFLP analysis

Sampled leaf material was placed into paper bags and dehydrated in silica gel. DNA was isolated from 10 mg of dried plant material of individual plants using the CTAB method (cetyltrimethylammonium bromide, Rogers and Bendich, 1994). Both DNA isolation and AFLP method (Vos et al., 1995) were adapted as previously described (Reisch, 2008; Reisch et al., 2005). DNA concentration was estimated photometrically and samples were standardised at a dilution of 7.8 ng/μl. For the AFLP procedure, genomic DNA (approximately 50 ng) was used for restriction and ligation reaction with MseI and EcoRI restriction enzymes and T4 DNA Ligase (both Fermentas) conducted in a thermal cycler for 2 h at 37 °C. Polymerase chain reactions (PCRs) were run in a reaction volume of 5 mL. Preselective amplifications were performed using primer pairs with a single selective nucleotide, MseI and EcoRI together with H_2O, Puffer S, dNTPs and Taq-Polymerase (PeqLab). The PCR reaction parameters were: 2 min at 94 °C, 30 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C, and 2 min of extension at 72 °C, followed by 2 min at 72 °C and ending with 30 min at 60 °C. After an extensive screening of selective primer combinations with eight randomly selected samples, selective amplifications were performed with the three primer combinations (MseI + CTA/EcoRI + ACC, MseI + CAC/EcoRI + AAG, MseI + CTC/EcoRI + ACA) and H_2O, dNTPs and Taq-Polymerase (PeqLab).

PCR reactions were performed with the touch–down profile: 2 min at 94 °C, ten cycles of 20 s of denaturing at 94 °C, 30 s of annealing, which was initiated at 66 °C and then reduced by 1 °C for the next ten cycles, 2 min of elongation at 72 °C, followed

Table 1
Sampled populations of *Nemesia barbata* with their geographic location, number of analysed individuals size of the fragment and genetic variation within populations measured as percentage of polymorphic loci and AMOVA derived SSWP/n-1 values. Fragments size following von Hase et al. (2003), estimations in field and from aerial photographs.

Nr.	Population name	Longitude (E)	Latitude (S)	N	Fragment size (ha)	PL	SSWP/n-1
1	Tygerberg	18°35′39″	33°52′37″	14	595	50.5	17.30
2	Kanonkop	18°36′16″	33°49′35″	7	78	35.9	15.62
3	Koeberg	18°33′28″	33°42′49″	13	141	34.6	11.90
4	Porquepine	18°35′15″	33°46′10″	8	248	30.1	13.32
5	Meerendal	18°37′23″	33°46′59″	12	298	48.5	16.17
6	Kanonkop 2	18°36′08″	33°49′05″	6	70	24.3	9.80
7	Sondagsfontein	18°39′44″	33°45′50″	5	78	20.9	10.10
8	Koopmanskoop 1	18°45′55″	33°54′14″	18	281	44.2	13.58
9	Zevenwacht	18°43′35″	33°55′16″	20	100	46.6	13.91
10	Mooiplaas	18°44′32″	33°55′29″	20	17	48.5	15.74
11	Wolf kloof 1	18°45′58″	33°54′53″	5	125	29.6	15.20
12	Wolf kloof 2	18°46′15″	33°55′17″	6	125	49.0	21.93
13	Koopmanskoop 2	18°46′58″	33°54′04″	6	7	34.5	15.77
14	Middlepos	18°38′37″	33°40′14″	20	4	53.4	17.13
15	Klipheuwel	18°41′23″	33°41′52″	20	52	59.7	19.80
16	Remshoogte 1	18°38′55″	33°38′33″	20	20	51.5	19.12
17	Helderfontein	18°42′52″	33°34′03″	10	100	45.2	19.39
18	Remshoogte 2	18°39′29″	33°38′51″	7	14	44.7	19.24
19	Klapmuts	18°44′45″	33°44′04″	3	34	27.2	18.67
20	Bonnie Doon	18°40′13″	33°39′56″	2	3	20.9	21.50

by 25 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C and 2 min of elongation at 72 °C, ending with a final extension for 30 min at 60 °C. After DNA precipitation, DNA pellets were vacuum-dried and dissolved in a mixture of Sample Loading Solution and CEQ Size Standard 400 (both Beckman Coulter). The fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analysed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Data were exported as crv-files, showing synthetic gels with AFLP fragments for each primer combination separately from all studied individuals and analysed in BIONUMERICS (Applied Maths, v. 3.0). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent. The error rate of AFLP analysis (Bonin et al., 2004) was estimated with 2.29%.

2.4. Statistical analysis

In the AFLP data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0. Finally, basic data structure consisted of a binary (0/1) matrix, representing the scored AFLP markers. Since the number of analysed individuals varied considerably between fragments we used AMOVA derived SSWP/n-1 values to characterize genetic variation within populations. We also calculated the percentage of polymorphic loci (PL) per population. Furthermore, the binary matrix was subjected to an analysis of molecular variance using GENALEX v. 6.2. Variance components (Φ_{PT}) and their significance levels for variation among populations and within populations were calculated. Moreover, a Principle Coordinate Analysis (PCoA) based on Bray Curtis distances was performed applying MVSP 3.12f (Kovach Computing Services, U.K.) to analyse the genetic relationship between individuals and populations. A Mantel test, based on 999 permutations, was conducted to test whether the matrix of pair-wise genetic distances, taken from the AMOVA between populations, was correlated with the matrix of geographical distances between populations (Mantel, 1967; Sokal and Rohlf, 1995).

3. Results

AFLP analyses revealed a total of 206 fragments: MseI-CTA/EcoRI-ACC (76 fragments), MseI-CAC/EcoRI-AAG (63 fragments), MseI-CTC/EcoRI-ACA (67 fragments). Percentage of polymorphic loci per population was highest in population Klipheuwel (59.7) and lowest in population Bonnie Doon (20.9). AMOVA derived SSWP/n-1 values ranged from 9.80 in population Kanonkop 2 to 21.50 in population Bonnie Doon (Table 1). Genetic variation within populations was, however, not correlated significantly with renosterveld fragment size or distance between fragments (Spearman correlation $p > 0.05$).

Table 2
Results of analysis of molecular variance of *Nemesia barbata*. Based on 206 AFLP fragments. Proportion of genetic variation (%). Significance level ($p > 0.001$) is based on 999 permutations. Degrees of freedom (D.f.).

Individuals/populations	Genetic variation	D.f.	Sums of squares	Means squares	%	Phi_{Pt}
222/20	Between populations	19	1653.111	87.006	29%	0.29
	Within populations	202	3279.308	16.234	71%	

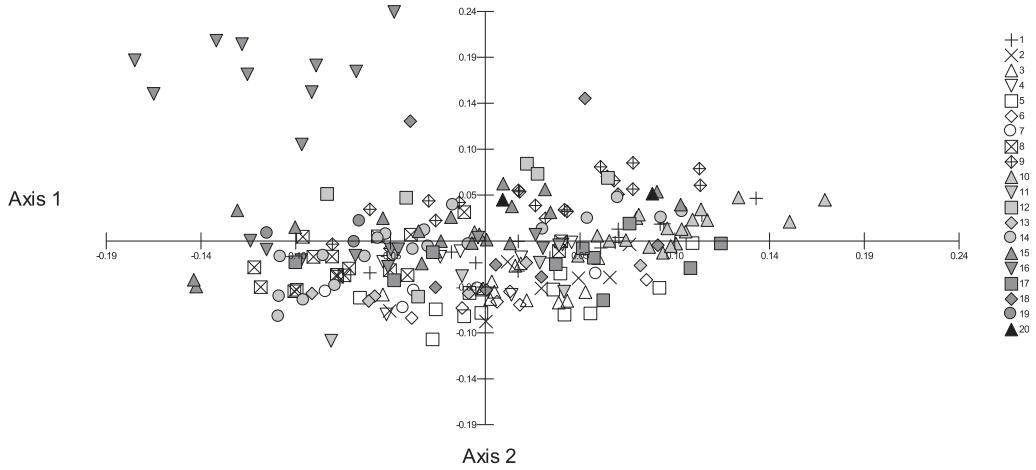

Fig. 1. Principal coordinates analysis of 222 *Nemesia barbata* individuals from 20 populations reveals weak differentiation between populations. Increment and Eigenvalues: Axis 1 (17.3%, 1.0), Axis 2 (12.0%, 0.7).

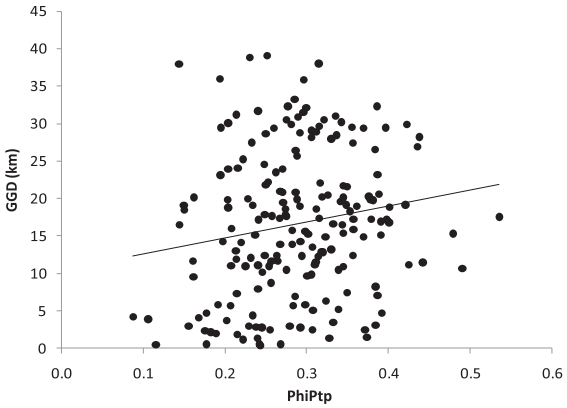

Fig. 2. Spatial genetic structure of *Nemesia barbata* populations in the south-western part of South Africa. Genetic variation between populations (Phi_{Pt}) and geographical distances (GGD; km) are positively correlated ($r = 0.166$, $p = 0.033$).

Analyses of molecular variance resulted in $Phi_{Pt} = 0.29$ (Table 2). However, PCoA analysis revealed only weak genetic differentiation and a large overlap between populations (Fig. 1). Mantel test showed a positive significant correlation of genetic (Phi_{Ptp}) and geographical distances between populations (GGD; km) for the entire dataset ($r = 0.166$, $p = 0.033$) (Fig. 2).

4. Discussion

Nemesia barbata is a common, mixed breeding annual herb occurring in low abundances in remnants of renosterveld vegetation. In this study, 222 individuals from 20 remnants were collected to characterize possible fragmentation effects on population genetics of the species.

We expected genetic pauperisation within small and isolated fragments and a lack of gene flow between these fragments. However, results revealed considerable genetic variation within, a low level of variation between populations and a positive correlation of genetic and geographical distances between populations. Furthermore, genetic variation within populations was neither correlated with the size of the renosterveld fragment nor with the distance to the nearest neighbour fragment.

Habitat fragmentation and subsequent smaller plant populations are susceptible to loss of genetic variation due to genetic drift (Honnay and Jacquemyn, 2007) and reduced gene flow (Young et al., 1996). However, this depends on species and site (Jacquemyn et al., 2003), as well as on landscape scale (Hutchison and Templeton, 1999). Two reasons might be responsible for the observed pattern of genetic variation and contradict the long fragmentation history of over a century and possible fragmentation effects on population genetic structure that should have been detected, especially in annual species with a short generation cycle. First of all, a genetically diverse and regularly activated soil seed bank could store much genetic variation (McCue and Holtsford, 1998) and buffer against genetic drift (Honnay et al., 2008). Secondly, although collapse of

pollination webs near urban areas are reported for the region (Pauw, 2007), it seems likely that pollen and/or seeds are able to bridge *N. barbata* populations, to ensure sufficient gene flow and avoids population differentiation (Slatkin, 1985). Seed dispersal is documented in fragmented European grassland species (Honnay et al., 2006), where livestock migrate between fragments. The small seeds of *N. barbata* are adapted to gravity-dispersal but could also move via endozoochorous dispersal via by migrating animals, thereby ensuring sufficient gene flow. However, in the case of restricted recent gene flow, observed genetic structure would be an imprint of historic conditions (Templeton, 1998). This shows that genetic theory of small populations does not always apply and ecological degradation is more severe for population persistence than genetic erosion (Kramer et al., 2008).

Therefore, little impact of fragmentation on genetic variation within and between *N. barbata* populations is currently visible. We suggest that a panmictic meta-population with random and erratic gene flow and no barrier for pollination and dispersal exists. More research is required on genetic variation of the soil seed bank, as well as pollination and dispersal vectors, in order to estimate entire genetic variation and current gene flow, respectively. Nevertheless, ongoing habitat transformation will result in population loss and needs to be avoided by the means of habitat protection.

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