New Phytologist

Spatial scale of local adaptation and population genetic structure in a miniature succulent, *Argyroderma pearsonii*

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Received: *27 November 2006*
Accepted: *20 January 2007*

Summary

• Explicit understanding of the spatial scale of evolutionary processes is required in order to set targets for their effective conservation. Here, we explore the spatial context of neutral and adaptive divergence in the species-rich Knersvlakte region of South Africa. Specifically, we aimed to assess the importance of erosional drainage basins as spatial units of evolutionary process.

• We used amplified fragment length polymorphism (AFLP) and reciprocal transplants to investigate genetic differentiation in *Argyroderma pearsonii*, sampled from sparse and dense quartz habitats within each of three drainage basins. This design allowed assessment of differentiation at two distinct spatial scales; between habitats within basins, and between basins.

• We found near-perfect concordance between genetic clusters and basin occupancy, suggesting restricted interbasin gene flow. In addition, transplants reveal adaptive divergence between basins on the dense quartz habitat.

• We have shown that neutral and adaptive differentiation occurs between basins, but not between habitats within basins, suggesting that conservation plans aimed at conserving multiple interconnected drainage basins will capture an important axis of evolutionary process on the Knersvlakte.

Key words: Aizoaceae, amplified fragment length polymorphism (AFLP), conservation genetics, local adaptation, population genetic structure, reciprocal transplant, South Africa.

New Phytologist (2007) **174**: 904–914

doi: 10.1111/j.1469-8137.2007.02043.x

Introduction

Empirical studies of spatially structured populations often reveal patterns of genetic and ecological divergence which are strongly correlated with geographical structure. This includes both adaptive responses to spatially structured variation in habitat parameters (Clausen *et al.*, 1940, 1948; Schemske, 1984; Galen *et al.*, 1991; Bennington & McGraw, 1995; Sambatti & Rice, 2006; Wright *et al.*, 2006) and neutral genetic divergence between geographically structured populations (Westerbergh & Saura, 1992; Mateu-Andres & Segarra-Moragues, 2000; Tribsch *et al.*, 2002; Tero *et al.*, 2003). Indeed, many population genetic processes (e.g. microenvironmental selection, clinal selection, genetic drift, gene flow and colonization) are interdependent with the spatial structure of the landscape (Epperson, 2003). An understanding of evolution in any natural system is thus contingent on an explicit appreciation of both the spatial scale and the spatial context of these microevolutionary processes.

In general, genetic drift and local adaptation in spatially structured populations are counteracted by the homogenizing effects of gene flow. The observed spatial structure of genetic differentiation results from the balance of these forces in space and through time (Wright, 1931; Slatkin, 1973; Kawecki &

Ebert, 2004). Spatial patterns of neutral and adaptive genetic differentiation need not be convergent as they primarily reflect the balance of different forces. Patterns of neutral genetic differentiation are readily discerned using molecular markers. Determining the adaptive significance of genetic differentiation is more difficult, particularly at the phenotypic level. One means to directly assess patterns of local adaptation is the use of reciprocal transplant experiments (Kawecki & Ebert, 2004). When these experiments are conducted at varying spatial scales and coupled with molecular assessment of spatial genetic structure, they can provide insight into the importance and scale of genetic drift, gene flow and natural selection as drivers of evolutionary divergence between populations.

Explicit understanding of the spatial scale of genetic differentiation and its cause has applied conservation value (Crandall *et al.*, 2000; Desmet *et al.*, 2002; Moritz, 2002). In recent years conservation efforts have begun to consider not only the genetic diversity within species (Frankel, 1974; Moritz, 1994, 1998) but also the evolutionary process that generates and maintains this diversity (Cowling *et al.*, 1999; Cowling & Pressey, 2001). For many systems, however, the relevant genetic data for conservation planning (i.e. the spatial structure of neutral and adaptive genetic variation) do not exist (Desmet *et al.*, 2002).

An understanding of population genetic structure may be key to conservation of the Succulent Karoo biome of South Africa, the earth's most biologically diverse arid system (Cowling & Hilton-Taylor, 1999). Leaf succulent members of the Aizoaceae have recently undergone an extensive radiation in the region, which in terms of pace and extent, rivals that of cichlids in the African rift lakes (Klak *et al.*, 2004). This single plant family accounts for a major portion (± 35%) of the biome's species diversity (Ihlenfeldt, 1994; Klak *et al.*, 2004). The Knersvlakte region of the Succulent Karoo is particularly diverse; it is only 100 × 80 km, yet is home to 129 endemic plant species (Hilton-Taylor, 1994). Most of these endemics are minute succulents, confined to the area's distinctive quartz lag gravel habitats (Schmiedel & Jürgens, 1999). These quartz habitat patches are associated with distinct drainage basins, separated from one another by an extensive matrix of non-quartz gravel soils. Restricted gene flow and local adaptation, as suggested by patterns of morphological variation and species turnover (Hartmann, 1977; Desmet *et al.*, 1998; Ellis *et al.*, 2006), may make each of these drainage basins discrete evolutionary units. Successful conservation efforts for the region may depend on an understanding of the evolutionary processes operating across this landscape (Desmet *et al.*, 1999, 2002).

We tested the 'drainage basin as evolutionary unit' hypothesis for *Argyroderma pearsonii*, an endemic miniature succulent species within the Aizoaceae. Specifically, we determined spatial population structure among basins from neutral genetic markers (amplified fragment length polymorphisms (AFLPs)) and tested for adaptation to soil variation within and between basins.

Materials and Methods

Study system and sampling design

Argyroderma N.E.Br. (Aizoaceae) is confined to the Knersvlakte region of the Western Cape Province of South Africa (30°45′–31°40′ S, 18°15′–19°00′ E). The Knersvlakte area (*c.* 100 × 80 km) comprises a highly eroded, deflating drainage system, consisting of a number of smaller erosion units associated with individual rivers which we refer to as drainage basins (Fig. 1). Removal of fine particles (silts and clays) from the soil profile by water runoff has resulted in the formation and continued expansion of extensive quartz gravel plains on the landscape surface, associated with individual drainage systems. The quartz habitat is not homogenous (Schmiedel & Jürgens, 1999), varying in both the characteristics of the quartz itself (e.g. pebble density, pebble size and pebble composition) and of the soil matrix (e.g. pH, salinity, soil depth and ionic composition). The edaphic heterogeneity of the area results from the diversity of quartz-intruded shales, phyllites and limestones of the Precambrian Nama group which underlie it (De Beer *et al.*, 2002).

Argyroderma pearsonii (N.E.Br.) Schw. is one of 11 species in the genus *Argyroderma* (Hartmann, 1977). It has a highly compact, stone-like growth form, usually comprising a single pair of leaves (Fig. 1. inset). It is confined to the quartz gravel plain habitat on which it often forms monodominant stands. Population densities vary considerably (17–249 individuals m^{-2}). Dense populations are generally found on substrates with a dense cover of quartz pebbles whereas quartz patches with sparse quartz pebble cover usually support lower densities of *A. pearsonii* individuals (dense quartz, 31 ± 16 adult individuals m^{-2}; sparse quartz, 5 ± 2 adult individuals m^{-2}). The landscape comprises a continuum of quartz pebble densities with patches of dense quartz usually surrounded by areas with sparse quartz cover. The high reflectivity of the quartz pebble layer may favour plant growth and survival by reducing soil surface temperatures and facilitating the nocturnal condensation of moisture on pebbles (Schmiedel & Jürgens, 2004), thus explaining the relationship between plant and pebble density.

Argyroderma pearsonii is obligately outcrossing (Hartmann, 1977; A. G. Ellis unpublished). The flowers are insect pollinated and are visited mainly by small solitary bees, small beetles and thrips (Struck, 1995; A. G. Ellis unpublished). Gene flow distances by pollen may thus be limited. *Argyroderma pearsonii* plants produce loculicidal, hygrochastic capsules which open to release seed when wet (Hartmann, 1977; Klak *et al.*, 2004). Fruit capsules, which are retained on the plant for many years, release seed during multiple rainfall events. The seed lacks inherent dormancy, with 100% of seed germinating during the first 4 d after wetting (Hartmann, 1977). Thus, primary seed dispersal distances are likely to be short and downstream, associated with rainfall runoff immediately following seed release during rainfall events.

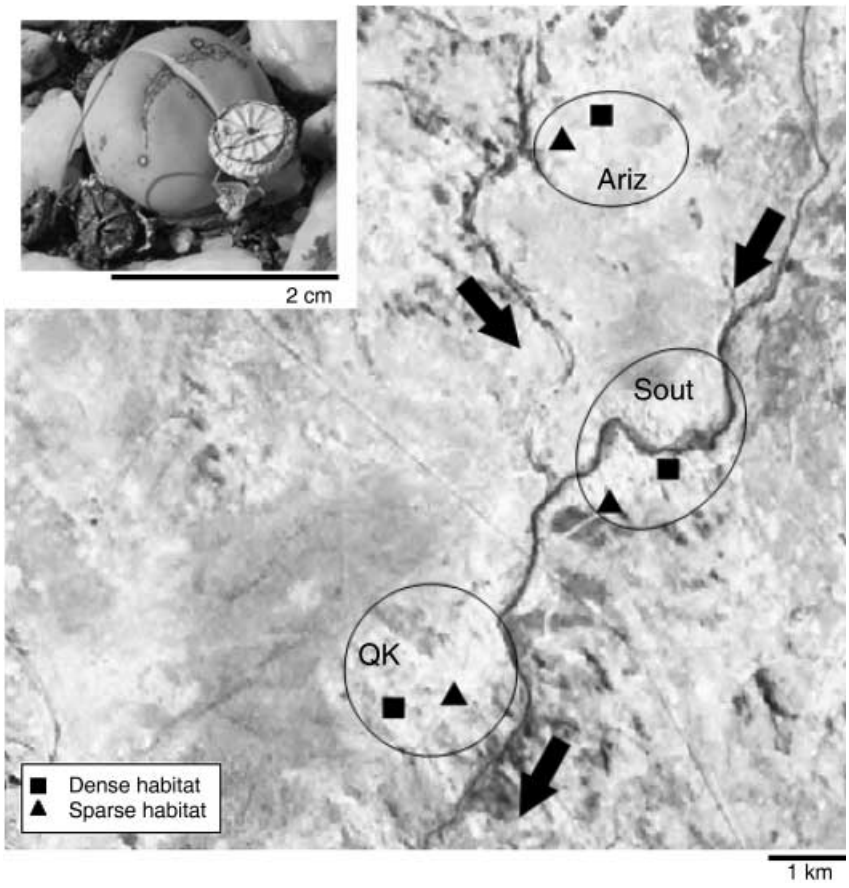

Fig. 1 Map showing the location of the six *Argyroderma pearsonii* populations sampled from three areas of quartz gravel plain habitat in three discrete drainage basin units. Squares, populations sampled on the dense quartz habitats; triangles, the sparse quartz habitats. Stream flow directions are indicated by arrows. The approximate area of quartz habitat in each drainage basin is encircled. Inset, photograph of an *A. pearsonii* individual with a typical loculicidal, hygrochastic capsule.

In this study we focus on six populations of *A. pearsonii* growing on quartz field habitat in three separate drainage basin units; Ariz, Sout and QK (Fig. 1). The Ariz basin is at the northern extreme of the distribution of *A. pearsonii*, the Sout basin is on the eastern extreme and the QK basin is at the centre of the species' distribution. The Ariz basin (along the Geelbeks tributary of the Sout river) is separated from the Sout and QK basins by a wide band of relatively quartz-free matrix habitat, whereas the Sout and QK basins are weakly connected by a series of quartz patches along the Sout river. The Sout basin sites were approximately 5 km from both the Ariz and QK basins, whereas the QK and Ariz basins were approximately 10 km apart. Within each drainage basin we sampled a pair of populations (0.4–1.2 km apart), one on habitat with dense quartz cover and the other on habitat with more sparse quartz cover (hereafter referred to as dense and sparse quartz habitat). This design allowed us to investigate the influence of both landscape structure (drainage basins) and habitat differences within basins on spatial genetic structure in the system.

Genetic structure

Between 10 and 20 widely spaced individuals were sampled from each population for AFLP analysis (Table 1). DNA was extracted from wet leaf material (100 mg) using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. Genomic DNA (*c.* 50 ng) was digested with *Mse*I and *Eco*RI (New England Biolabs, Ipswich, MA, USA) restriction enzymes and then ligated to double-stranded *Eco*RI and *Mse*I adapters. The restriction–ligation reactions were incubated overnight at room temperature. Ligate was then preamplified using the PE Applied Biosystems (Foster City, CA, USA) Ligation and Preselective Amplification Module. Preselective polymerase chain reactions (PCRs) were run using 10 µl reaction volumes. A subsample of eight individuals was screened with 40 selective primer pairs using the Selective Amplification Start-up Module for Small Genomes (PE Applied Biosystems). Four selective primer pairs (E.TG/M.CTT, E.AA/M.CAC, E.AC/M.CAT., E.AG/M.CTA), which produced clear, reproducible bands and displayed variation between and within populations, were selected for subsequent analyses. Selective PCRs were performed using 10 µl reaction volumes. Multiple AFLP reactions were independently performed on several samples for internal control. The fluorescently labelled selective PCR products were separated on 5% denaturing Long Ranger gels using an ABI Prism 377 DNA Sequencer. Genescan-500-ROX (PE Applied Biosystems) was used as an internal lane size standard. Raw data was extracted and bands were sized using GENESCAN analysis

Table 1 Genetic diversity indices for the six *Argyroderma pearsonii* populations sampled, as well as cumulative indices for each drainage basin

Basin	Habitat	*n*	Bands/individuals	%P	H_e	Rare	Private
Sout	Dense	15	205.4 ± 2.4	47.6	0.177 ± 0.008	7.1 ± 0.6	6
	Sparse	13	208.1 ± 1.7	45.4	0.175 ± 0.008	7.6 ± 0.8	3
QK	Dense	14	210.1 ± 2.0	47.6	0.182 ± 0.008	7.4 ± 0.8	7
	Sparse	14	212.8 ± 2.0	48.4	0.184 ± 0.008	8.8 ± 0.8	7
Ariz	Dense	20	209.1 ± 1.6	53.4	0.181 ± 0.008	6.8 ± 0.5	7
	Sparse	10	209.7 ± 4.0	57.1	0.194 ± 0.009	7.0 ± 0.6	6
Sout		28	206.6 ± 1.5	53.6	0.181 ± 0.008	7.3 ± 0.5	12
QK		28	211.5 ± 1.4	55.8	0.189 ± 0.008	8.1 ± 0.6	23
Ariz		30	209.3 ± 1.7	56.5	0.192 ± 0.008	6.8 ± 0.4	25

n, Number of individuals sampled; Bands/individual, mean number of bands scored per individual ± SE; %P, percentage of polymorphic bands at the 5% level; H_e, Nei's gene diversity ± SE; Rare, rare bands (< 10% of all individuals) per individual ± SE; Private, bands confined to a single population or drainage basin.

software (PE Applied Biosystems). Genescan files were then imported into Genotyper (PE Applied Biosystems) which was used to score fragments across all individuals. Potential AFLP loci were initially located automatically by searching for peaks higher than 500 florescence units within the size range 50–500 base pairs (bp), but scoring across all individuals for each fragment was done manually. Fragments were excluded from the analysis if the standard deviation in fragment size across individuals was > 0.2 bp or if peak heights varied substantially across individuals. Results were exported as a binary, presence/absence matrix for subsequent analyses.

Four measures of genetic diversity within populations were estimated from the AFLP dataset. The percentage of polymorphic loci (P%) and Nei's (1987) unbiased expected heterozygosity (H_e) were calculated using the Lynch & Milligan (1994) square-root method of allele frequency estimation implemented in AFLPsurv (Vekemans *et al.*, 2002). In addition the number of private alleles (bands confined to a single population) and the number of rare alleles per individual (bands present in < 10% of all individuals) were estimated for each population.

The structure of genetic differentiation in the system was investigated using three methods. First, we used the Bayesian clustering algorithms implemented in the program STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003) to determine objectively the number of clusters present in the AFLP dataset and to determine to what extent these genetic clusters correspond to basins, habitats or populations. We used an admixture model which allows individuals to have mixed ancestry and assumed correlated allele frequencies (i.e. that allele frequencies of clusters are random draws from the same distribution). Models which did not allow admixture and which assumed uncorrelated allele frequencies yielded qualitatively similar results. We used multiple runs of 20 000 burn-in and 30 000 data collection MCMC iterations to determine the posterior Bayesian probabilities of the existence of one through six clusters in the dataset. We used the membership coefficients (Qs) to determine each individual's estimated membership fraction in each of the inferred clusters.

Second, we estimated global F_{st} values across all six populations using the Lynch & Milligan (1994) approach for dominant markers as implemented in AFLPsurv (Vekemans *et al.*, 2002). Significance was tested against a null model of no structure by randomly permuting individuals among populations. Effective gene flow between basins was estimated indirectly from the global interbasin F_{st} estimate (Wright, 1931). Third we used molecular analysis of variance (AMOVA, Excoffier *et al.*, 1992) to investigate the hierarchical structuring of genetic variation in the system. Total genetic variance was partitioned in two ways: among drainage basins, among populations (habitats) within drainage basins and within populations; and between habitats (dense and sparse), among populations within habitat and within populations. This allowed assessment of the spatial structure of molecular variance in the system (i.e. between drainage basins and between the sparse and dense quartz habitats).

Edaphic characterization of habitats

Soil pH, electrical conductivity, soil stone content and quartz pebble cover were determined for all six *A. pearsonii* populations. These variables were chosen to characterize edaphic habitats, as they have been shown by Schmiedel and Jürgens (1999) to be important predictors of plant community composition on the Knersvlakte. Five bulked soil samples, each consisting of three 1000-cm^3 soil cores (10 cm deep), were collected within each population. Soil was passed through a 1 mm sieve and the stone content was calculated by weight. Electrical conductivity (mS cm^{-1}) was measured through a soil paste and pH was determined in a 0.1 M KCl solution at Matrolab, Cape Town, South Africa. Quartz pebble cover was estimated from three digital photographs (*c.* 1 × 0.5 m of surface) taken at midday within each population. We used colour recognition software to estimate the proportion of the ground surface covered by the highly reflective white quartz pebbles. These estimates are conservative in that pebble surfaces in shadow are not included and should thus not be viewed as absolute, but rather

used in a comparative sense. The proportion of pebbles greater than 1 cm diameter was also estimated from photographs and this value was then combined with the cover values to give an estimate of the cover of larger (> 1 cm) pebbles. Univariate two-way factorial ANOVAs (PROC GLM, SAS Institute, 2001) were used to assess edaphic variation between drainage basins and between the dense and sparse quartz habitats. Significance levels of the four tests were Bonferroni adjusted accordingly.

Local adaptation

Three sets of experiments, each comprising three reciprocal transplants were performed as follows: between habitats (dense and sparse quartz habitat) within each drainage basin; between drainage basins on the dense quartz habitat only; and between drainage basins on the sparse quartz habitat only. We did not conduct transplants between dense and sparse habitats in different basins and, as a result, each garden did not contain plants from all six sites. Instead, each appropriately randomized transplant garden comprised 18 individuals from each of four locations: one group from within the same population (control), two groups from the same habitat type in the other two basins (interbasin transplants) and a fourth group from the other habitat type within the same basin (interhabitat transplant).

Seventy-two adult individuals in their first year of reproduction (i.e. plants with no fruits retained from previous years) were collected from each population during July 2000. Soil was removed from the roots of all individuals which were then measured, aged and randomly assigned to four groups of 18 individuals each. The following day individuals were planted into randomly assigned positions on 1.25×1 m transplant grids within each population in the design described earlier. The transplant sites had previously been cleared of existing vegetation. The transplant grids were watered for 2 wk after transplanting in order to facilitate establishment and minimize transplant shock. Each year from 2001 until the transplants were removed in 2003, survival and size (length and width) of each individual was recorded in November, at the end of the growing season. In order to prevent genetic contamination of natural populations, all transplant grids were covered in a fine mesh cage for the duration of flowering each year. The transplant experiments were terminated after 3 yr for the same reason. Plants were hand-pollinated with pollen from outside the grid, resulting in fruit-set in all cases regardless of their basin of origin. Thus, fruit set measurements effectively measured the ability of plants to produce flowers and were not influenced by possible accumulation of genetic incompatibilities between basins.

We assessed local adaptation in the three reciprocal transplant experiments through three surrogate measures of fitness. These were growth (size increase of survivors from 2000 to 2003), survival (survived or died) and reproduction (0, 1 or 2 fruits per survivor). The growth data were analysed using GLM models with homesite (site of origin) and testsite (transplant site) as fixed factors. Survival and reproduction data were analysed using generalized linear models (PROC GENMOD, SAS Institute, 2001) with homesite and testsite as categorical variables. Models of the survival data were constructed assuming an underlying binomial distribution and using the logit link function, whereas reproduction was treated as an ordinal response variable and analysed using a multinomial distribution and the cumulative logit link function. Preliminary analyses showed that initial size of transplanted individuals influenced survival and reproduction and it was thus included as a factor in all analyses.

For the between-habitat (within-basin) experiment, the treatment effects included basin (QK, Sout and Ariz), homehabitat (dense, sparse) and testhabitat (dense, sparse). A significant testhabitat × homehabitat interaction term provides evidence for global local adaptation to habitats within basins, whereas the basin × test × home interaction term tests whether the degree of local adaptation to habitat varies among basins.

For the interbasin transplants on dense and sparse quartz habitats, the treatment effects included homebasin and testbasin (QK, Ariz and Sout). The home × test interaction terms from these analyses, which potentially indicate local adaptation to drainage basins, were difficult to interpret because of the three-way transplant design used. To overcome this we ran a priori contrasts to test for significant fitness differences (1) between plants transplanted into their home basin and other basins (home vs away) and (2) between local plants and foreign plants within each drainage basin (local vs foreign). In addition, contrasts were constructed for analyses to test for differences between test habitats and test basins.

Results

Genetic structure

The final genetic matrix consisted of 496 AFLP loci (91% polymorphic) scored across 86 individuals. All individuals had unique AFLP profiles with an average of 209.1 (SE 0.9) fragments per profile. All populations exhibited similar levels of genetic polymorphism (45.4–57.1%) and had similar numbers of rare and private alleles (Table 1). Estimates of gene diversity and rare alleles were similar for drainage basins, but QK and Ariz had twice as many private alleles as the Sout basin (Table 1).

Bayesian clustering analysis yielded an unambiguous estimate of $K = 3$ clusters in the AFLP dataset. The Bayesian posterior probability of $K = 3$ given the data was ϕ1.0, whereas posterior probabilities for $K = 1, 2, 4, 5$ and 6 ranged between 8.53E-17 and 2.1E-257. The three clusters correspond very closely to the drainage basin units (Fig. 2). Only three individuals in the QK sparse site and one individual in the Sout sparse site were ambiguously assigned to their drainage basin cluster (Fig. 2) and may thus be migrants or the products of gene flow between populations. Individuals from Ariz and from the Sout dense site exhibited virtually no admixture,

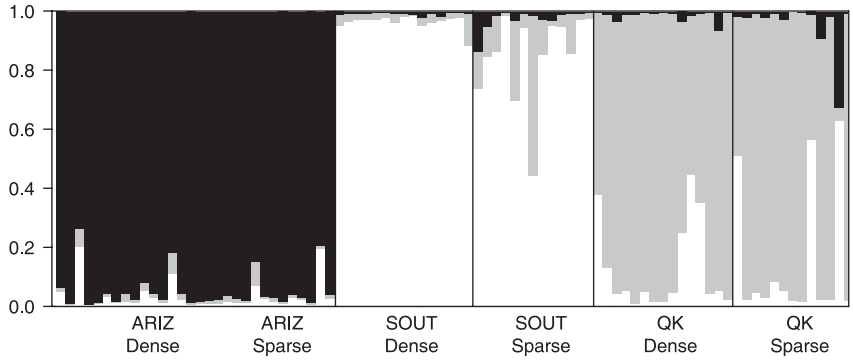

Fig. 2 Plot illustrating the membership of sampled *Argyroderma pearsonii* individuals in each of three genetic clusters (white, grey and black) objectively identified from the amplified fragment length polymorphism (AFLP) dataset using the Bayesian clustering algorithms implemented in the program STRUCTURE. Vertical bars represent individuals and shaded segments represent their estimated membership percentage in each of the three inferred clusters. Individuals are ordered according to their population of origin (i.e. basin name (Ariz, Sout or QK) and habitat type (dense or sparse)). The genetic clusters clearly correspond to drainage basin occupancy. Stream flow direction is from left to right (Ariz–QK).

Table 2 Hierarchical molecular analysis of variance (AMOVA) partitioning variance in the amplified fragment length polymorphism (AFLP) dataset between drainage basins and between habitats

	df	SS	VC	%V	*P*
Drainage basins					
Among basins	2	317.573	3.076	5.83	0.065
Among populations within basins	3	206.783	1.506	2.85	< 0.001
Among individuals within populations	80	3857.167	48.215	91.32	< 0.001
Total	85	4381.523	52.797		
Habitats					
Among habitats	1	77.530	–0.832	–1.60	0.752
Among populations within habitat	4	446.827	4.481	8.64	< 0.001
Among individuals within habitat	80	3857.167	48.215	92.96	< 0.001
Total	85	4381.523	51.864		

df, Degrees of freedom; SS, sum of squares; VC, variance component; %V, percentage of genetic variation at each hierarchical level.

Table 3 Pairwise ϕ_{st} estimates of genetic divergence between *Argyroderma pearsonii* populations from molecular analysis of variance (AMOVA; below the diagonal) and geographic distances (km) between populations

	Ariz Dense	Sparse	Sout Dense	Sparse	QK Dense	Sparse
Ariz						
Dense		0.38	5.01	4.70	8.98	8.61
Sparse	0.0122		5.33	4.99	8.33	7.96
Sout						
Dense	0.0915	0.0662		0.92	5.32	4.27
Sparse	0.0807	0.0616	0.0124		4.43	3.43
QK						
Dense	0.0818	0.0721	0.0426	0.0237		1.19
Sparse	0.0917	0.0661	0.0333	0.0153	0.0123	

whereas some individuals with mixed ancestry were present in the QK basin and the Sout sparse site.

Argyroderma pearsonii exhibited significant population genetic structure. The global F_{st} estimate ($F_{st} = 0.052$) was low, but significantly different from zero at the 99% confidence level. Hierarchical AMOVAs show that whereas 5.83% of variance in the AFLP dataset was partitioned among drainage basins, habitat explained no genetic variance (Table 2). The majority of genetic variance occurs among individuals within populations (91%). Populations within a drainage basin were most similar ($\phi_{st} = 0.012$ for all drainage basins – Table 3). Pairwise comparisons between QK and Sout populations had intermediate ϕ_{st} values (0.015–0.043) whereas the highest levels of genetic divergence occurred between the Ariz populations and the rest (ϕ_{st}: 0.062–0.092, Table 3).

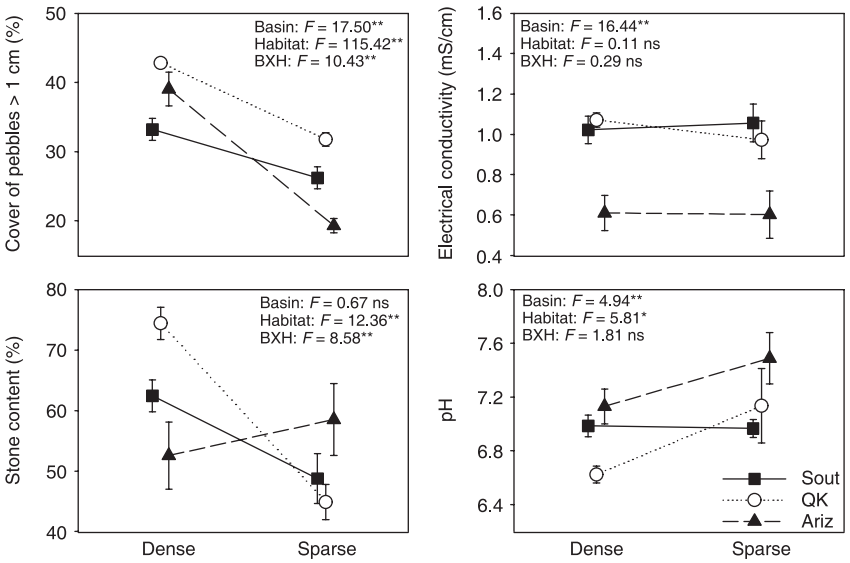

Fig. 3 Soil characteristics (pH, electrical conductivity, stone content and quartz pebble cover) measured in *Argyroderma pearsonii* populations from dense and sparse quartz habitats in three drainage basins (squares, Sout; circle, QK; triangles, Ariz). Population means (± SE) as well as *F*-statistics for main (basin and habitat) and interaction effects from two-way ANOVAS are shown for each variable. Quartz cover and stone content differ significantly between habitats, whereas conductivity and quartz cover differ between basins. **, Significant after Bonferroni adjustment; *, significant before Bonferroni adjustment; NS, not significant.

Edaphic characterization of habitats

Analysis of soil variables confirms that the dense quartz habitats occupied by *A. pearsonii* have significantly higher soil stone content and quartz pebble cover than sparse quartz habitats (Fig. 3). In addition to variation between habitat types within drainage basins, we found significant variation between the edaphic environments of the drainage basins sampled. Drainage basins differ significantly in terms of electrical conductivity (ionic concentrations) and quartz cover (Fig. 3). Soil pH was significantly correlated with electrical conductivity ($r = -0.47$, $P < 0.05$).

Local adaptation

In this study we found no evidence for local adaptation at the between-habitat level (dense vs sparse quartz habitats). Neither the homehabitat × testhabitat, nor the basin × home × test, interaction terms were significant for any of the three fitness surrogates (Table 4). Instead, we found that generally the sparse habitats are inferior, leading to lower survival and reproduction (Table 4, Fig. 4). This habitat effect is not consistent between basins, however, as indicated by the significant basin × test interaction terms for growth, survival and reproduction (Table 4). In the QK basin growth rates and fruit production are higher in the sparse quartz habitat and in the Sout basin survival is higher in the sparse habitat.

Similarly, the interbasin transplants on the sparse quartz habitat also yielded no evidence for local adaptation (Table 5). Instead, we found a significant testbasin effect in analyses of all fitness components. Growth and reproduction are significantly reduced in the Ariz basin. In addition, plants from the Ariz basin had significantly reduced fruit production across all sites.

Table 4 Results from generalized linear models of fitness components from interhabitat transplants

	Growth		Survival		Reproduction	
	F	*P*	χ^2	*P*	χ^2	*P*
Basin	6.1	**	0.4		83.6	***
Home	0.23		0.11		4.11	*
Test	1.24		4.68	*	6.36	*
Basin × home	0.9		0.44		4.54	
Basin × test	5.18	*	10.57	*	20.89	***
Home × test	0.77		0.4		0.86	
Basin × home × test	0		0.35		4.44	
Size	6.31	*	0		14.49	***

Basin, drainage basin (Sout, QK, Ariz) in which the *Argyroderma pearsonii* transplants were performed; Home, habitat of origin (Dense, Sparse); Test, habitat into which transplants were conducted. The lack of significant home × test and basin × home × test interaction terms indicates no local adaptation in these transplants. Size was included as a covariate in all analyses.
*, **, ***, Significant at $P < 0.05$, $P < 0.005$ and $P < 0.0005$, respectively.

By contrast, the interbasin transplants on the dense quartz habitat did yield evidence for local adaptation to individual drainage basins. We found a significant homebasin × testbasin interaction effect on reproduction (Table 5). Specifically, there was a significant increase in reproduction of local vs foreign genotypes in the Sout and QK basins, although not in the Ariz basin (Table 6). In addition comparisons of performance between plants transplanted into their home basin and those transplanted into other basins revealed that Sout plants had significantly higher growth and reproduction in their home basin, QK plants had significantly higher survival at home and Ariz plants had significantly higher reproduction in their home basin (Table 6, Fig. 5).

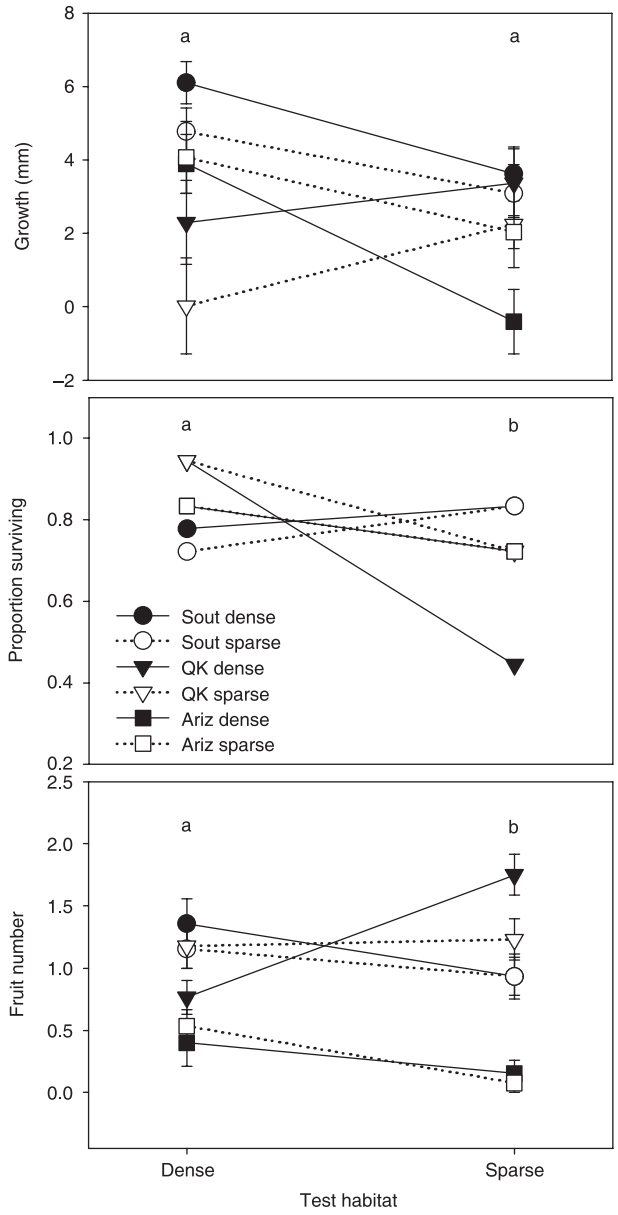

Fig. 4 Reaction norms for *Argyroderma pearsonii* growth, survival and reproduction from reciprocal transplants between the dense (closed symbols) and sparse (open symbols) habitats (circles, Sout; triangles, QK; squares, Ariz) within drainage basins. Lines represent the home site or population of origin and the *x*-axis represents the test site. Letters represent significant differences between test habitats from a priori contrasts.

Table 5 Results of generalized linear models of fitness components from interbasin transplants on dense and sparse quartz habitat

	Growth		Survival		Reproduction	
	F	*P*	χ^2	*P*	χ^2	*P*
Dense habitat						
Size	4.44	*	3.90	*	7.76	*
Home	0.23		4.40		42.10	***
Test	9.30	***	6.70	*	2.34	
Home × test	0.92		7.57		13.70	*
Sparse habitat						
Size	20.72	***	1.27		6.57	*
Home	1.01		0.79		23.81	***
Test	3.18	*	9.56	*	24.48	***
Home × test	0.90		4.92		2.65	

Home, site of origin; Test, site into which *Argyroderma pearsonii* transplants were conducted; interaction term (home × test) tests for potential local adaptation. Initial size of transplanted individuals was included as a covariate in all analyses.
*, **, ***, Significant at $P < 0.05$, $P < 0.005$ and $P < 0.0005$, respectively.

Table 6 Results of a priori contrasts from generalized linear models of interbasin transplants on dense quartz habitat, performed to test for significant differences in fitness components: between *Argyroderma pearsonii* plants transplanted into their home basin and other basins (home vs away); and between local plants and foreign plants within each basin (local vs foreign)

	Growth		Survival		Reproduction	
	χ^2	*P*	χ^2	*P*	χ^2	*P*
Home vs away						
Sout	7.00	*	2.14		13.5	***
QK	7.41		9.67	**	0.04	
Ariz	1.78		0.03		4.19	*
Local vs foreign						
Sout	1.18		0.05		20.45	***
QK	0.05		5.47	*	12.27	***
Ariz	0.26		1.87		11.90	

Results are shown for growth and survival although interaction terms in models for these fitness components were not significant.
*, **, ***, Significant at $P < 0.05$, $P < 0.005$ and $P < 0.0005$, respectively.

Discussion

This study supports the hypothesis that drainage basin units are an important spatial component of evolutionary process on the Knersvlakte. We found near-perfect correspondence between assignment of individuals to genetic clusters based on the AFLP dataset and drainage basin occupancy (Fig. 2), which suggests that gene flow between basins is restricted. Although significant, the level of genetic divergence between drainage basins is low ($\phi_{st} = 0.02$–0.09). Indirect estimates of gene flow between basins ($Nm = 3.39$) are high enough (i.e. $Nm > 1$) to suggest that substantial genetic differentiation between basins through genetic drift alone is unlikely (Wright, 1931; Slatkin & Barton, 1989). However, the product Nm measures effective gene flow and thus confounds the effects of gene flow, genetic drift and even local adaptation through time (Hutchison & Templeton, 1999). Estimates of Nm could be high despite low levels of pollen and seed movement

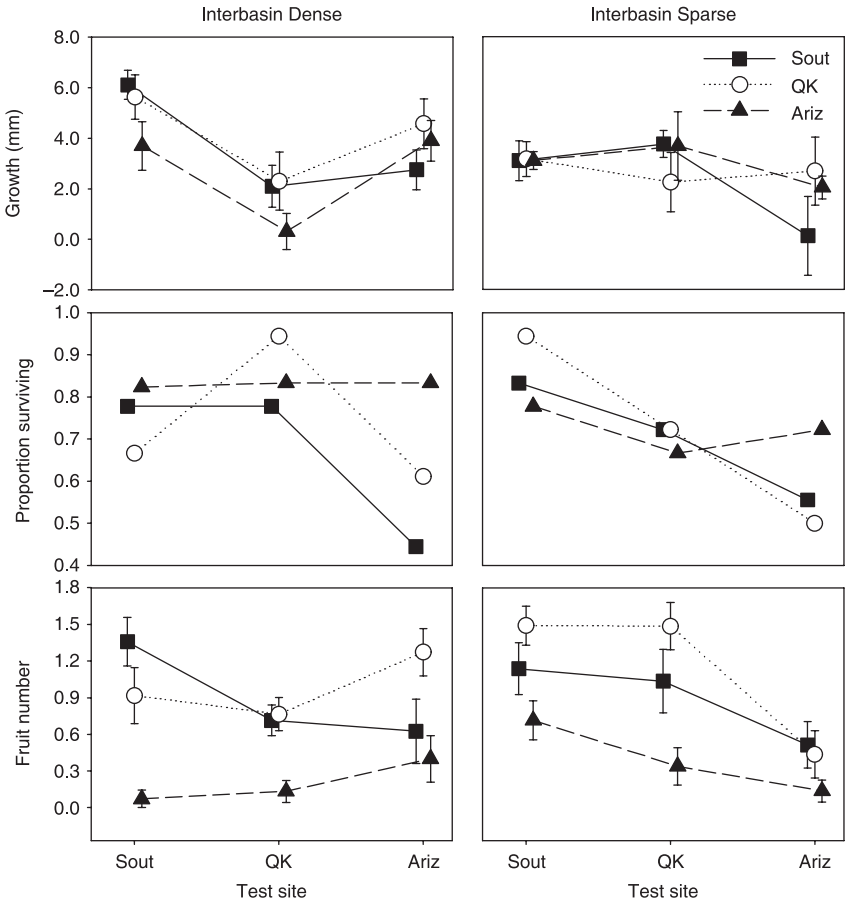

Fig. 5 Reaction norms for *Argyroderma pearsonii* growth, survival and reproduction from reciprocal transplants experiments between drainage basins on both dense and sparse quartz habitat (squares, Sout; circles, QK; triangles, Ariz). Lines represent the home site or population of origin and the *x*-axis represents the test site. The homebasin × testbasin interaction term is only significant for reproduction (fruit number) in the interbasin dense transplants.

between basins if genetic drift in populations is weak (i.e. effective population sizes are high). We found little variation between populations in genetic diversity estimates or in numbers of rare and private alleles (Table 1), perhaps suggesting that they have similar demographic and evolutionary histories. Estimates of genetic diversity within populations of *A. pearsonii* are equivalent to, although slightly lower than, those reported for other perennial, outcrossing, late successional plant species (Nybom, 2004), although direct comparison between studies is limited by differences in sampling, techniques and analyses used (Lowe *et al.*, 2004).

The patterns of genetic admixture revealed by the Bayesian clustering analysis (Fig. 2), coupled with levels of divergence between populations (Table 3), further support the importance of restricted gene flow in generating spatial structure in the system. Genetic differentiation between basins appears to reflect ecological isolation of basins and not just isolation by distance (Table 3). For example, populations in the Ariz basin, which is most isolated ecologically (i.e. occurs along a separate river, is separated by nonquartz habitat and has most distinct soils; Figs 1, 3), are most diverged genetically (Table 3) and show little evidence of admixture with either the Sout or QK populations (Fig. 2). By contrast, the Sout sparse site and the downstream QK sites show lower levels of genetic divergence and a higher incidence of admixed individuals, demonstrating the connectedness of these two basins (Fig. 2). Interestingly, the Sout dense site, which is furthest upstream (Fig. 1), shows no admixture with the QK sites (Fig. 2). The increasing incidence of admixed individuals in a downstream direction along the Sout river supports the idea that seed movement by water runoff may result in directional gene flow and thus some level of connectedness between drainage basins along stream courses (Desmet *et al.*, 1998). However, the presence of admixed individuals in the Sout sparse site also suggests some upstream gene flow.

Unlike the moderate, but clear-cut pattern of divergence between basins we found very low levels of neutral genetic differentiation between populations on different habitats within a basin (Tables 2 and 3). Although the dense and sparse quartz habitats within basins differed in terms of pebble cover and stone content (Fig. 3), reciprocal transplant experiments detected no adaptive divergence between populations on these habitat types (Table 4). Instead, the sparse quartz habitat was generally inferior for the survival and reproduction of *A. pearsonii* (Table 4, Fig. 4), perhaps attesting to the important role of the dense quartz layer in facilitating growth and survival of these miniature plants (Schmiedel & Jürgens, 2004).

Although we found no evidence for adaptive divergence between the sparse and dense habitats within basins, reciprocal

transplants suggest that adaptive divergence has occurred between populations in different drainage basins. Transplants between basins on the dense quartz habitat yielded significant interaction effects for reproduction, indicating that populations on this habitat type are locally adapted to the unique edaphic environment in their home basins (Table 5). The pattern of the homebasin × testbasin interaction for fitness in transplants involving the Sout and QK dense populations meets both criteria proposed by Kawecki & Ebert (2004) as diagnostic of local adaptation (i.e. significantly increased performance of local vs foreign genotypes within basins and increased fitness in the home basin relative to other basins) (Table 6). Evidence for local adaptation of the Ariz population is weaker.

While local adaptation occurs on the dense quartz habitat, reciprocal interbasin transplants provide no evidence for local adaptation to the sparse quartz habitat (Table 5). One potential explanation for this contradictory pattern is simply that statistical power is reduced for these contrasts because the sparse habitat is generally of poorer quality. However, this result may also point to real differences in adaptive regime between sparse and dense habitats, owing to habitat quality differences and demographic processes operating within drainage basins. Theoretical models suggest that adaptive divergence between habitats is hindered by differences in the quality and size of habitats (Kawecki, 1995; Kisdi, 2002) as well as by asymmetric gene flow between habitats (Holt & Gaines, 1992; Kawecki, 1995; Holt, 1996). The majority of *A. pearsonii* populations occur on the higher-quality dense quartz habitat which supports higher population densities than the sparse quartz habitat. In addition low-density populations on the sparse quartz habitat may be sustained by propagule flow from neighbouring populations on dense quartz habitat in a classic source–sink metapopulation structure. Under this scenario the majority of individuals within a basin are exposed to the selective regime imposed by the dense quartz habitat, making local adaptation to this habitat most likely. Furthermore, any selection in favour of novel mutations with adaptive advantage in the sparse habitat would be swamped by gene flow from upstream neighbouring dense populations (Holt & Gaines, 1992; Kawecki, 1995; Holt, 1996). This would account for the low levels of genetic divergence between dense and sparse habitats within basins and could also impede local adaptation to the sparse habitats across all basins. Reciprocal transplants between sparse and dense habitats in different drainage basins are needed to confirm this proposed mechanism.

This study clearly indicates the importance of drainage basins as relevant spatial units of both neutral and adaptive variation within *A. pearsonii* on the Knersvlakte. Systematic conservation plans, which explicitly aim to conserve multiple interconnected drainage basin units, are thus likely to capture an important axis of the evolutionary processes driving diversity patterns in the area (Desmet *et al.*, 1999, 2002). Reserve design efforts should also aim to maximize edaphic habitat variation between selected basins in order to adequately capture the scale of divergent selection gradients in the system. Although, we find no evidence for genetic structure within drainage basins, a previous study did find evidence for local adaptation of parapatric *Argyroderma* species occupying different habitats within drainage basins (Ellis & Weis, 2006), suggesting that habitat diversity should also be an important criterion for assigning conservation priority to basins.

The interconnectedness between landscape structure and population genetic processes illustrated in this study supports the idea that restricted gene flow coupled with the topographic and geological heterogeneity of the Succulent Karoo has been important in the explosive evolutionary radiation of the Aizoaceae (Ihlenfeldt, 1994). Our demonstration that in *A. pearsonii* gene flow between basins is restricted over short distances (*c.* 5 km) and adaptive responses to edaphic heterogeneity may be occurring over similar spatial scales, represents the first direct evidence that supports this claim.

Acknowledgements

We thank Boetie Louw and Buys Wiese for permission to work on their property, Boetie Louw for his generous hospitality in the field, Amy Spriggs, Graeme Ellis and Benjamin Klaus for their assistance in the field, the Leslie Hill Institute for Plant Conservation for providing a base in Cape Town. Diane Campbell, Richard Cowling, Timm Hoffman, Nely Pohl, Mark Rausher and six anonymous reviewers provided useful comments on previous versions of this manuscript. The Western Cape Nature Conservation provided permits to conduct the work and financial support was provided by the National Research Foundation, South Africa.

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