

# Coexistence and differentiation of ‘flowering stones’: the role of local adaptation to soil microenvironment

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## Summary

**1** In most documented examples of adaptive radiation, the processes driving divergence of phenotypes and, ultimately, speciation remain speculative. The remarkable radiation of the Aizoaceae in the southern African winter-rainfall deserts is no exception.

**2** We examined the role of specialization to soil microenvironments in the coexistence and differentiation of the aizoaceous genus *Argyroderma*, which is confined to quartz-lag gravel habitat in southern Africa.

**3** We examined patterns of edaphic habitat use and morphological divergence between three sympatrically occurring species (one habitat generalist and two quartz specialists) and used transplant experiments to investigate the extent of local adaptation to quartz microenvironments.

**4** Soils utilized by populations of the generalist, *A. fissum*, and one of the specialists, *A. pearsonii*, differ significantly in terms of stone content and quartz cover. The soils utilized by the two specialist taxa (*A. pearsonii* and *A. delaetii*) do not differ significantly in terms of the variables measured, although *A. pearsonii* consistently uses habitats with higher quartz cover at all contact zones investigated.

**5** The three species are clearly separated morphologically. The morphological trend between *Argyroderma* species occupying sparse (*A. fissum*) and dense (*A. pearsonii* and *A. delaetii*) quartz habitats is towards reduction in plant size and height (dwarfism), increased levels of submergence, decreased branching, spherical leaf shapes, increased leaf thickness and increased enclosure of the leaf by the old leaf sheaths. The two specialist taxa differ in terms of leaf sheath enclosure, plant submergence and fruit traits.

**6** Transplant experiments demonstrate home-site survival advantage in transplants between species occupying sparse and dense quartz habitats as well as between microenvironments within the dense quartz habitat.

**7** The results suggest that divergence in potentially functional morphological traits between *Argyroderma* species occupying different edaphic microenvironments probably results from local adaptation, with coexistence facilitated by response to fine-scale habitat variation. In addition, the results suggest a possible role for edaphic specialization in the evolutionary divergence of *Argyroderma* and perhaps in the broader radiation of the Aizoaceae in southern Africa.

**Key-words:** Aizoaceae, coexistence, edaphic specialization, local adaptation, reciprocal transplant experiment, South Africa, Succulent Karoo

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## Introduction

Adaptive radiations occur when a single ancestor diverges into numerous species that occupy a variety of environments and differ in traits important for the utilization of these environments (Schluter 2000). The study of adaptive radiation thus requires two things: (i) to demonstrate radiation of species and phenotypes from a common ancestor and (ii) to demonstrate a direct role for divergent natural selection in diversification. Demonstrating significant correlation between genetically based phenotypic traits and environmental features represents a first step towards fulfilling the second of these requirements. In addition, in order to eliminate the possibility of adaptively neutral phenotype–environment associations, it is essential to demonstrate that these traits enhance fitness (Arnold 1983; Wainwright 1994).

The classical reciprocal transplant experimental design (Clausen *et al.* 1940) has been widely used to test the association between phenotype and fitness in different environments. When phenotypic trait optima differ between environments due to divergent natural selection the resulting trade-offs will lead to variation in the fitness of a particular phenotype across environments, which can be detected by transplanting. In plants this approach has been used to show performance differences of phenotypically differentiated ecomorphs, populations or closely related species across climatic (Ehleringer & Clark 1988), altitudinal (Galen *et al.* 1991), topographic (Bennington & McGraw 1995), soil (Fritsche & Kaltz 2000), salinity (van Zandt *et al.* 2003; Beaton & Dudley 2004), heavy metal (Lehmann & Rebele 2004) and biotic (Schoen *et al.* 1986) gradients. In addition to revealing mechanisms of divergence, transplant experiments can also tell us about the mechanisms controlling coexistence and range limits in communities of closely related species (Connell 1961; Antonovics 1976; Sultan 2001). In particular, they reveal the extent to which the distribution of a species is dictated by an adaptive fit between phenotype and environment.

The species-rich winter-rainfall desert plant communities of the Succulent Karoo in southern Africa are dominated by leaf succulent members of the Aizoaceae (Cowling & Hilton-Taylor 1999). Approximately 1563 species within the Ruschioid subfamily of the Aizoaceae, ranging in growth form from miniature succulents (stone plants) to large shrubs, have arisen in the last 8.7–3.8 million years (Klak *et al.* 2004). This represents the most remarkable radiation reported from the plant kingdom, in terms of both the tempo of evolution and the extent of the radiation. By any measure the ruschioid radiation meets the first criterion for demonstrating the occurrence of adaptive radiation. However, evidence pertaining to the second criterion – demonstrating a role for divergent natural selection – is limited, although the diversity of species and growth forms within the group has been attributed to complex interactions

between the availability of numerous diverse habitats and high levels of genetic isolation between populations (Ihlenfeldt 1994).

In this study we use transplant experiments to investigate the fit between phenotype and soil microenvironment in *Argyroderma*, a genus within the ruschioid subfamily of the Aizoaceae. *Argyroderma* consists of 11 taxa, all of which are confined to the Knersvlakte region of the Succulent Karoo, an area approximately 100 × 80 km in extent (Hartmann 1978). The genus has diversified onto the regionally unique quartz-gravel plain habitat that characterizes the Knersvlakte (Schmiedel & Jurgens 1999). Ten taxa are quartz specialists, exhibiting highly reduced growth forms associated with the quartz gravel plain habitat to which they are confined. The eleventh taxon, the generalist, is highly branched and mat-forming, occurring on both quartz gravels and quartz-poor matrix habitat. Species within the genus are distinguished by very few discrete characters. The majority of morphological traits vary continuously within the genus. Hartmann (1978) suggested that morphological differentiation occurs in response to edaphic variation across the Knersvlakte, in particular along gradients of quartz pebble density and soil salinity.

In a parallel study, investigating the influence of landscape, habitat and flowering time on patterns of genetic (AFLP) and morphological differentiation across the genus, Ellis *et al.* (in press) found evidence for spatial genetic isolation at all taxonomic levels. In contrast, morphological differentiation occurs along a habitat axis, between populations occupying different edaphic microenvironments. Morphological divergence is not associated with geographical isolation and genetic (AFLP) distance does not account for a significant proportion of the variance in morphological distance between specialist populations. This evidence suggests that patterns of morphological differentiation within *Argyroderma* do not reflect neutral processes. Instead the correlation between soil and morphology probably arises through local adaptation to soil microenvironments, although it may also result from plastic phenotypic responses to edaphic variation.

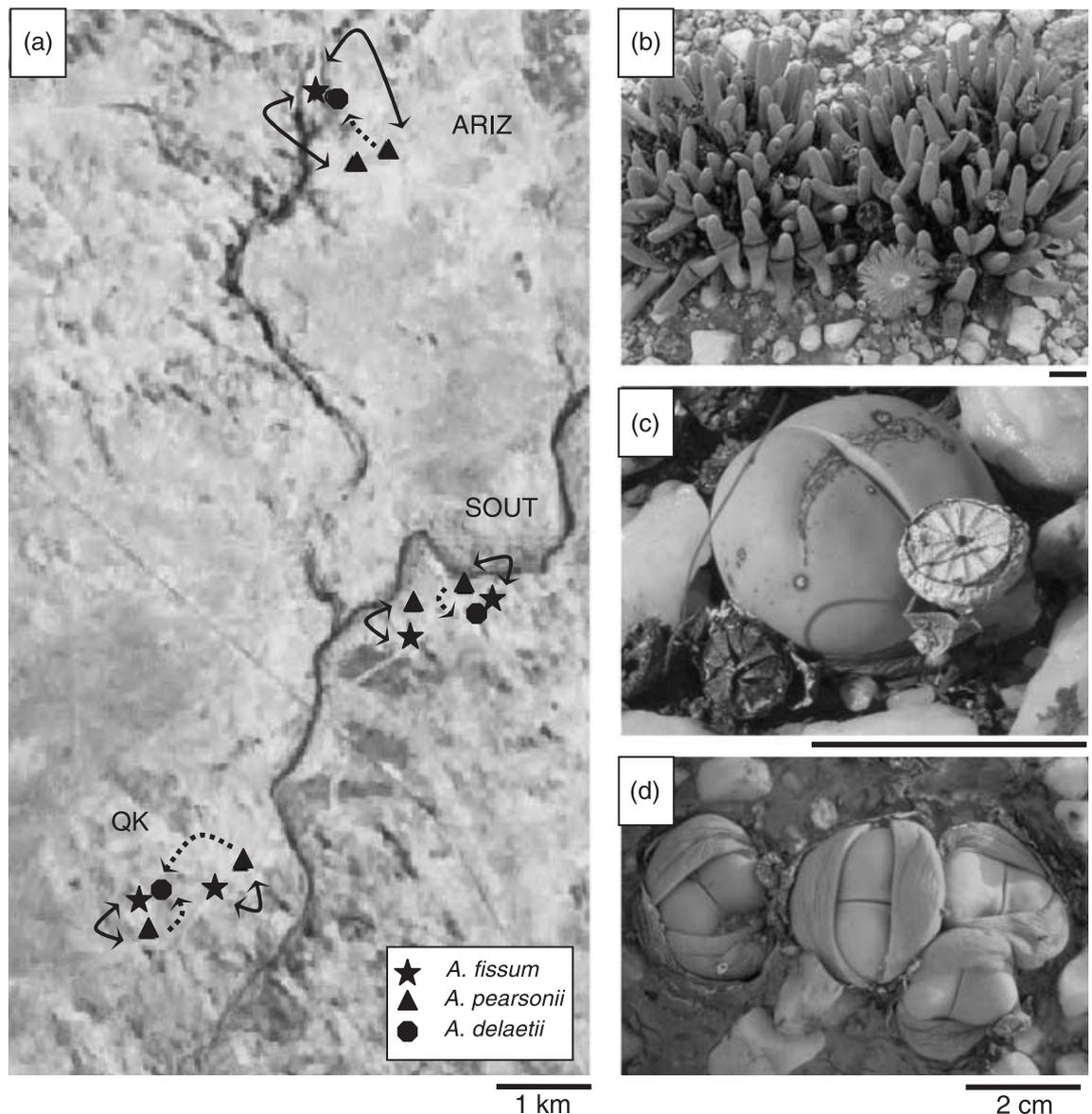
This study aimed to distinguish between these two possibilities. First, we describe morphological and habitat use divergence between three coexisting species of *Argyroderma* and then use transplant experiments to determine whether species are locally adapted to the specific edaphic microenvironments they inhabit. Specifically we ask: Do coexisting *Argyroderma* species utilize different soil microhabitats? Do coexisting species exhibit divergence in potentially functional morphological traits? Are species locally adapted to soil conditions? Does the evidence suggest a role for adaptive morphological differentiation, both across the quartz habitat boundary (between sparse and dense quartz habitats) and between microenvironments within the quartz habitat, in determining patterns of coexistence and differentiation in *Argyroderma*?

## Materials and methods

### STUDY AREA

*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 area (c. 100 × 80 km) is bounded by the Olifants River valley in the south, the steep sandstone scarp of the Cape Folded Belt in the east, granitic–gneiss uplands in the north and the Atlantic Ocean in the west. The area is underlain by heterogeneous sediments of the Precambrian Nama Group, predominantly shales, phyllites and limestones all of which are heavily intruded by quartz veins (De Beer *et al.* 2002). The Knersvlakte

area comprises a highly eroded, deflating drainage basin, consisting of a number of smaller erosion units associated with individual river systems (Fig. 1a). 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 homogeneous, varying in the characteristics of both the quartz itself (e.g. pebble density, pebble size, pebble composition) and the soil matrix (e.g. pH, salinity, soil depth, ionic composition). Schmiedel & Jurgens (1999) described two distinct quartz-field edaphic habitat types which represent the extremes of a habitat continuum and support separate plant communities. On the one extreme are



**Fig. 1** (a) Map showing the location of transplant arenas within populations sampled from three drainage basins. Symbols represent species. Solid arrows are reciprocal transplants between *A. pearsonii* and *A. fissum* populations. Transplants back into the population of origin were performed in each case. (b) *A. fissum*, the generalist. (c) *A. pearsonii*, the subglobose quartz specialist. (d) *A. delaetii*, the submerged quartz specialist. Scale bars in b–d represent 2 cm. Photographs by A. G. Ellis.

quartz-fields with high salt content, neutral pH and lower stone content which represent the most edaphically arid habitat, and on the other extreme are soils with high stone content, low pH and lower salt content.

#### STUDY SPECIES

*Argyroderma* is a genus of compact, perennial dwarf succulents with growth forms which range from single-bodied sunken forms through branched mat-forming species (Smith *et al.* 1998). *Argyroderma* species are obligately outcrossing and flowers are cup-like, pollinated primarily by small solitary bees (Struck 1995; A. G. Ellis, unpublished data). Reproductive isolation between sympatric species appears to be largely achieved through separation of flowering phenologies (Hartmann 1978; Ellis *et al.* in press). The three species studied were the generalist *A. fissum* (Haw.) L. Bol., and two quartz specialist taxa, *A. pearsonii* (N.E.Br.) Schw. and *A. delaetii* Mass. The highly branched generalist, *A. fissum* (Fig. 1b), is thought to represent the ancestral growth form in the genus (Hartman 1978). A survey of AFLP variation within the genus revealed a well-supported basal split between *A. fissum* and a largely unresolved polytomy, including all 10 quartz specialist taxa (Ellis *et al.* in press). Although the AFLP survey was unable to resolve relationships between specialist taxa, it did suggest that the specialists are divided into two largely non-overlapping sets of genotypic clusters, which correspond to two groups of species differing in morphology and habitat use. The stone-like *A. pearsonii* and *A. delaetii* (Fig. 1c,d) studied here belong to different genotypic clusters within the specialist clade.

These three species occur in sympatry or 'near parapatry' at all three of the drainage basins studied (ARIZ, SOUT and QK, Fig. 1). This pattern of coexistence (i.e. the generalist and one species from each specialist genotypic cluster) occurs throughout the range of the genus. Distribution patterns at any site are complex, with turnover between virtually monodominant patches of each species occurring over very small spatial scales (*c.* 1–100 m; Schmiedel & Jurgens 1999). *A. pearsonii* is truly sympatric (i.e. grows interspersed) with the other species in only a very small proportion of its range, usually in narrow contact zones between monodominant patches. *A. fissum* and *A. delaetii*, however, grow interspersed at two of the study sites and throughout the joint range of *A. fissum* and the '*A. delaetii*' species group.

#### MORPHOLOGICAL CHARACTERIZATION OF SPECIES

Three growth form (whole plant) characteristics were measured for between 4–14 reproductive individuals in each of six *A. pearsonii*, four *A. fissum* and three *A. delaetii* populations. These traits were plant diameter (mean horizontal diameter of each plant), plant height (height of the plant above the soil surface) and

plant submergence (plant height above the soil surface minus the average leaf height). Seven leaf characteristics were measured on three leaf pairs per individual, where possible, and character averages were calculated for each individual. Leaf traits measured were: leaf pair length (distance across the leaf pair), leaf height (distance from the base to the tip of a leaf), leaf thickness (distance from the ventral to the dorsal leaf surface), leaf pair shape (leaf width divided by leaf pair length), leaf gap (distance between leaf tips divided by the leaf pair length), proportion of the leaf contained within the sheath (height of leaf above sheath divided by the leaf height) and leaf sheath persistence (count of annually produced leaf sheaths which are retained on each leaf pair). Five fruit characteristics were measured on up to three fruits per plant and then averaged to generate fruit trait measurements for each individual. The fruit characters measured were: capsule diameter (average fruit diameter), capsule shape (shortest diameter divided by the longest fruit diameter), locule number (number of carpels), bract length (length of the bract subtending the fruit) and the degree of enclosure of the capsule by the bract (shortest distance between the bract tip and the rim of the capsule).

Principal components analysis (PCA – SAS, SAS Institute 2001) was used to assess the clustering of individuals in multivariate morphological space. Individuals were clustered on the basis of 15 normally distributed morphological characters. Percentage data were arcsine-transformed for analysis. Plant diameter, plant height, leaf pair length, leaf thickness and leaf height data were positively skewed and heteroscedastic and were thus log-transformed before analysis. Component scores were calculated and plotted on the first two principal component axes. Factor loadings (eigenvectors) on the first two axes were used to evaluate the contribution of individual traits to discrimination of species in multivariate morphological space. Mean population factor scores along the first and second axes were calculated for comparison with soil characteristics measured within each population.

#### EDAPHIC CHARACTERIZATION OF HABITATS

Soil pH, electrical conductivity, soil stone content and quartz pebble cover were determined for parapatric populations of *A. fissum* and *A. pearsonii* at each of five sites in three drainage basins. In addition, *A. delaetii* populations were sampled at three of these sites (Ariz, QK1 and Sout1). The variables chosen to characterize edaphic habitats were those shown by Schmiedel & Jurgens (1999) to be important predictors of plant community composition in the Knersvlakte. Between three and 10 bulked soil samples, each consisting of three 1000-cm<sup>3</sup> soil cores (10 cm deep), were collected within each population. Soil was passed through a 1-mm-mesh sieve and the stone content was calculated by weight. Electrical conductivity (mS cm<sup>-1</sup>) was measured through a soil paste and pH was determined in

a 0.1 M KCl solution at Matrolab, Cape Town, South Africa (pH – Beckmann® 43, EC – Metrohm E527). Ammonium acetate (1 M) extractions were used to determine the available ionic concentrations of Na, P, Ca, Mg, K, Cu, Mn and Zn to plants for a single sample from each of six *A. pearsonii* and four *A. fissum* sites at Matrolab, Cape Town (Aurora A1500 AA spectrophotometer). Quartz pebble cover was estimated from three digital photographs (*c.* 1 m × 0.5 m of surface) taken at midday within each population. We used colour recognition software (ColourRange – available from the authors upon request) 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 in 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 mixed-model factorial ANOVAS (as implemented in PROC GLM in SAS, SAS Institute 2001) with site as a random factor were used to determine whether the habitats utilized by *A. fissum* and *A. pearsonii* or *A. delaetii* and *A. pearsonii* differ consistently in terms of pH, conductivity, stone content and quartz cover. Both stone content and quartz cover were arcsine-transformed prior to analysis. A priori contrasts were performed between species at each of the sites. In order to compare soil and morphological divergence between species, a principal components analysis was used to extract a single 'soil' axis accounting for the maximum possible variance in measurements of pH, conductivity and stone content between samples. And *t*-tests were used to assess whether *A. fissum* and *A. pearsonii* habitats differ significantly in terms of the exchangeable cations and micronutrients measured.

#### TRANSPLANT EXPERIMENTS

Two transplant experiments were conducted: the main experiment involved reciprocal transplants between the generalist, *A. fissum*, and a specialist, *A. pearsonii*; a smaller experiment involved transplanting *A. pearsonii* into the habitat of another quartz specialist, *A. delaetii*. The full complement of transplants between these three species was not feasible as *A. delaetii* plants had released their seed prior to arrival at the field site. In all cases transplants were conducted between species within a geographical location (Fig. 1a).

In December 1999 fruit capsules were collected from approximately 30 plants each of *A. pearsonii* and *A. fissum* at six sites where populations of the two species occur in close parapatry. Seed was extracted from all fruits (*c.* 100–1500 seeds per fruit) and seed from the 30 mothers within each species-by-site combination was combined. After thorough mixing the seed was divided into groups of exactly 100 seeds in preparation for sowing.

The following year during the rainy season (June 2000) seed was planted into transplant arenas at each of six sites (11 transplant arenas – Fig. 1a). Transplant arenas consisted of 25 20 × 20 cm plastic seedling pots (transplant cells) from which the bases had been removed. To prevent water runoff from removing transplant seed or introducing foreign seed, the bottomless pots were sunken into the soil so that 3–5 cm of plastic was exposed above the quartz surface. Pots were placed approximately 10 cm apart to form a 1.5 × 1.5 m square transplant arena. Care was taken not to disturb the quartz pebble layer when doing this. All *Argyroderma* plants were removed from within 1 m of the arena to prevent dispersal into the arenas (van Rooyen *et al.* 1980). To eliminate the soil seed bank, the arenas were watered for 1 week and then thoroughly weeded prior to transplanting. One hundred seeds were sown into each of three randomly allocated, replicate transplant cells per treatment. To ensure germination, transplant cells were watered daily for 10 days. The seeds have no inherent dormancy and glasshouse trials show that all viable seed (*c.* 80%) germinate within 10 days of watering. In May 2003, 3 years after sowing, the experiments were harvested. All quartz was carefully removed from each transplant cell and all surviving individuals were collected. The number and mean mass of surviving individuals per transplant cell was determined.

The empty cells within each arena served as a control for dispersal into the arenas and for movement of seeds between cells. Some dispersal into the transplant arenas was detected (*c.* three seedlings per cell). It was possible, using data from mapped demographic monitoring plots in each population, to determine the minimum size attained by plants in the population that had germinated in 2000. All plants within empty cells were smaller than this minimum size, and thus we concluded that dispersal into the plots had occurred during 2001 and 2002 but not during 2000. This conclusion was further supported by the fact that very little natural recruitment occurred in any population during 2000, but that large amounts occurred subsequently, especially during 2002. Only plants greater than the cut-off size (*i.e.* plants which had germinated in 2000) were used in the analyses.

Mean survival and mass (growth rate) data from the three replicate cells per treatment at each of the six sites in the *A. fissum* – *A. pearsonii* experiment served as input data for two-way factorial ANOVAS with home site (species) and test site (environment) as fixed effects. The data were  $\ln(x + 1)$ -transformed prior to analysis. In particular we were interested in the significance of the interaction term (species × environment), which would indicate environment-specific performance differences (survival and growth) and thus provide evidence for local adaptation in the system. We performed two a priori contrasts between survival and growth rate in non-native and native habitats for each of the test species.

In addition, at four of the six sites *A. pearsonii* seed was transplanted into arenas established within populations of another specialist species, *A. delaetii*. This experiment was conducted as described above, although it was not reciprocal, in that *A. delaetii* was not transplanted into the *A. pearsonii* arenas. To determine whether *A. pearsonii* performed differently in these two habitat types we used a GLM model with test site (environment) as a fixed factor and population of origin as a random effect. In this analysis a significant main effect of environment indicates reduced fitness in the non-native habitat.

The strength of local adaptation for each transplanted species was estimated as the reduction in fitness (survival and growth) in the foreign environment relative to the home environment. Specifically, we tested the expectation that the magnitude of the transplant effect would be greater for the specialist than for the generalist species. We used paired *t*-tests to compare the magnitude of the transplant effect (absolute and percentage reduction in survival and growth rate) between the specialist, *A. pearsonii*, and the generalist, *A. fissum*, as well as between *A. pearsonii* seed transplanted into *A. fissum* and *A. delaetii* habitat.

## Results

### MORPHOLOGICAL CHARACTERIZATION OF SPECIES

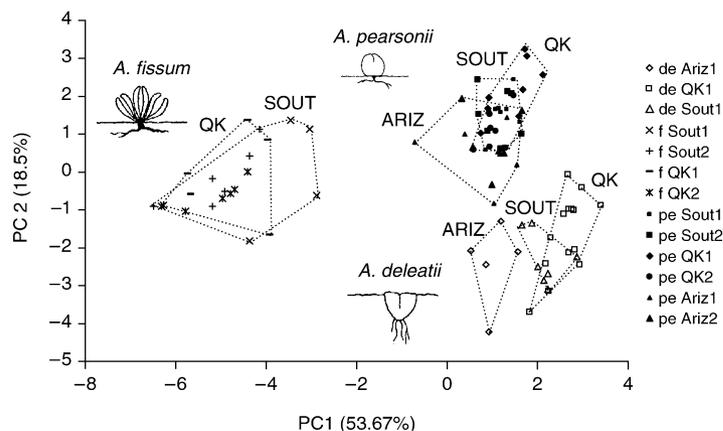
Individuals of the three species are clearly separated on the first two axes of a PCA of 15 morphological traits (Fig. 2). The first axis, which separates *A. fissum* from the two quartz specialist taxa, accounts for 53.67% of the variance in the dataset. A number of growth form and leaf traits load negatively on this axis, namely plant diameter, leaf length, plant height, leaf gap width and submergence. Leaf shape, leaf thickness and the proportion of the leaf enclosed within a sheath all load positively on the first principal component. In addition,

capsule size, bract size and distance between the bract and the capsule load negatively on the first axis. The second axis of the PCA, along which the quartz specialist taxa (*A. pearsonii* and *A. delaetii*) are separated, accounts for a further 18.5% of the variance in the data (Fig. 2). The length across the leaf pair (leaf size) and the proportion of the leaf enclosed within a sheath load negatively on this axis, whereas the persistence of the leaf sheaths loads positively. In addition, capsule traits (number of locules, capsule size, capsule shape and fruit bract length) load negatively on this axis. Populations of the same species from different drainage basins are not morphologically distinct, with the possible exception of the *A. delaetii* population from the Ariz basin (Fig. 2).

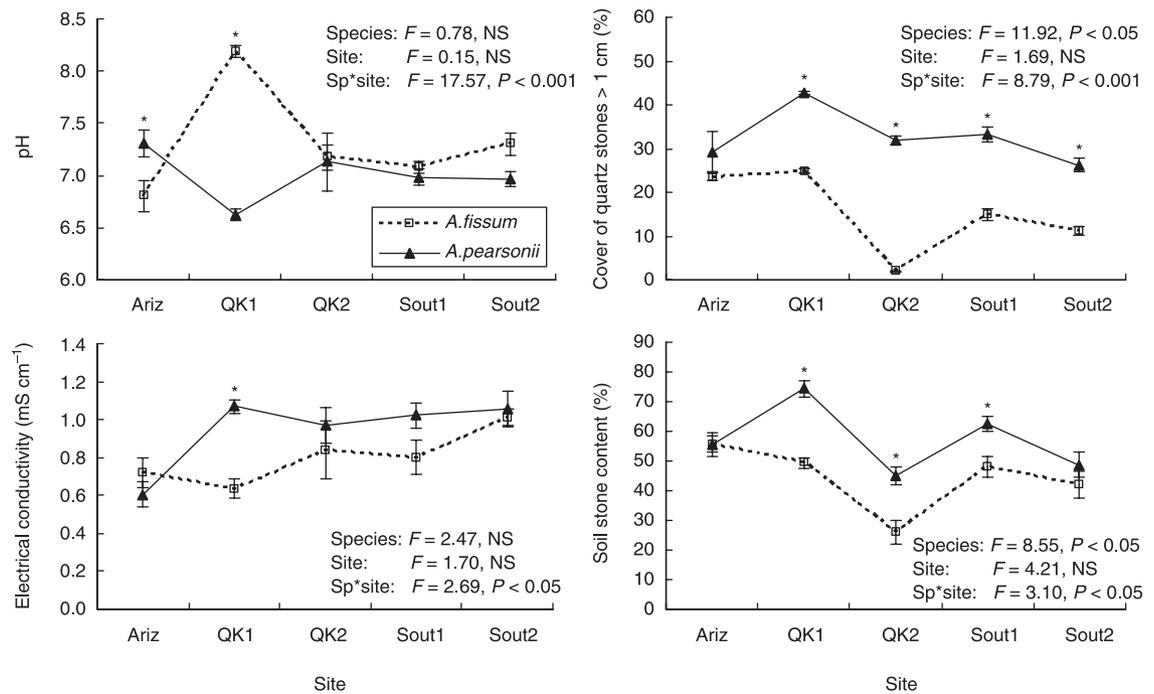
### EDAPHIC CHARACTERIZATION OF HABITATS

Soil stone content and quartz cover differ significantly between the habitats utilized by the generalist, *A. fissum*, and a specialist, *A. pearsonii* (Fig. 3). The quartz habitat specialist earns this title as it occurs on soils with significantly higher quartz cover and stone content (Fig. 3). Although pH and conductivity do not differ significantly between *A. pearsonii* and *A. fissum* overall, pH tends to be higher in generalist populations and conductivity lower (Fig. 3). The difference between soils occupied by each species is not constant between sites, as indicated by the significant interaction term (species  $\times$  site) in all analyses (Fig. 3). Manganese was the only nutrient which differed significantly in concentration between the habitats of *A. fissum* and *A. pearsonii* (Table 1).

Habitats utilized by the two specialist species, *A. pearsonii* and *A. delaetii*, did not differ significantly across sites for any measured soil variables (Fig. 4). However, a priori contrasts suggest that quartz cover differed significantly between species' habitats at each individual site, with *A. pearsonii* always occupying habitats with more dense quartz cover (Fig. 4). Again,



**Fig. 2** The separation of *A. fissum* (generalist), *A. pearsonii* and *A. delaetii* in multivariate morphological space. Axes represent the first two principal components from a PCA analysis of 15 morphological traits. Polygons have been drawn around individuals sampled from separate drainage basins (Ariz, QK and Sout). Illustrations represent the growth form of each species. de, *delaetii*; f, *fissum*; pe, *pearsonii*.



**Fig. 3** Soil characteristics (pH, conductivity, stone content and quartz cover) measured in paired parapatric populations of *A. fissum* and *A. pearsonii* at five sites. Means and standard errors as well as  $F$ -values for main (species and site) and interaction effects from mixed-model ANOVAs are shown for each variable. Soil stone content and surface quartz cover differ significantly between *A. fissum* and *A. pearsonii* habitats. Asterisks indicate significant a priori contrasts ( $P < 0.05$ ) between species at a given site.

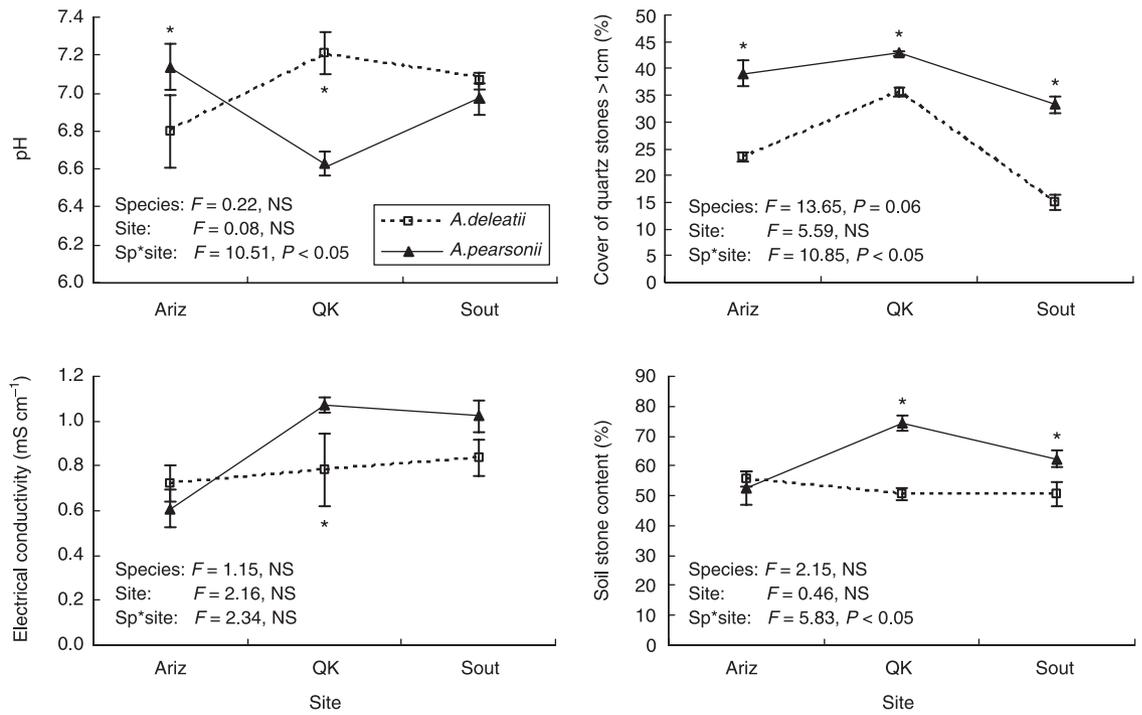
**Table 1** Soil variables (stone and pH, salinity indicators, fertility indicators and toxic metals) measured in populations of *A. pearsonii* ( $n = 6$ ) and *A. fissum* ( $n = 4$ ). Means and ranges (in parentheses) are shown for each species. The  $t$  column shows significance of  $t$ -tests comparing species means for each variable (except for pH, stone content, pebble cover and conductivity where  $P$ -values for the species main effect from two-way mixed-model ANOVAs are shown)

	<i>A. fissum</i>	<i>A. pearsonii</i>	$t$
pH	7.35 (6.5–8.4)	7.05 (6.4–7.9)	NS
Soil stone content (%)	43 (13–60)	57 (34–80)	$P < 0.05$
Pebble cover (%)	15 (2–26)	32 (18–44)	$P < 0.05$
Conductivity (mS cm <sup>-1</sup> )	0.81 (0.24–1.14)	0.89 (0.28–1.33)	NS
Na ( $\mu\text{g g}^{-1}$ )	2679 (1670–3500)	3304 (2060–4300)	NS
P ( $\mu\text{g g}^{-1}$ )	93 (51–183)	60 (22–172)	NS
Ca ( $\mu\text{g g}^{-1}$ )	836 (61–1361)	476 (209–684)	NS
Mg ( $\mu\text{g g}^{-1}$ )	631 (520–750)	910 (289–1955)	NS
K ( $\mu\text{g g}^{-1}$ )	210 (113–280)	217 (130–334)	NS
Ca : Mg ratio	1.3 (0.1–1.97)	0.8 (0.2–1.85)	NS
Cu ( $\mu\text{g g}^{-1}$ )	1.8 (0.9–2.3)	2.8 (1.9–5.6)	NS
Mn ( $\mu\text{g g}^{-1}$ )	224 (160–310)	314 (270–360)	$P < 0.05$
Zn ( $\mu\text{g g}^{-1}$ )	1.4 (0.9–1.9)	4.2 (1.6–8.9)	NS

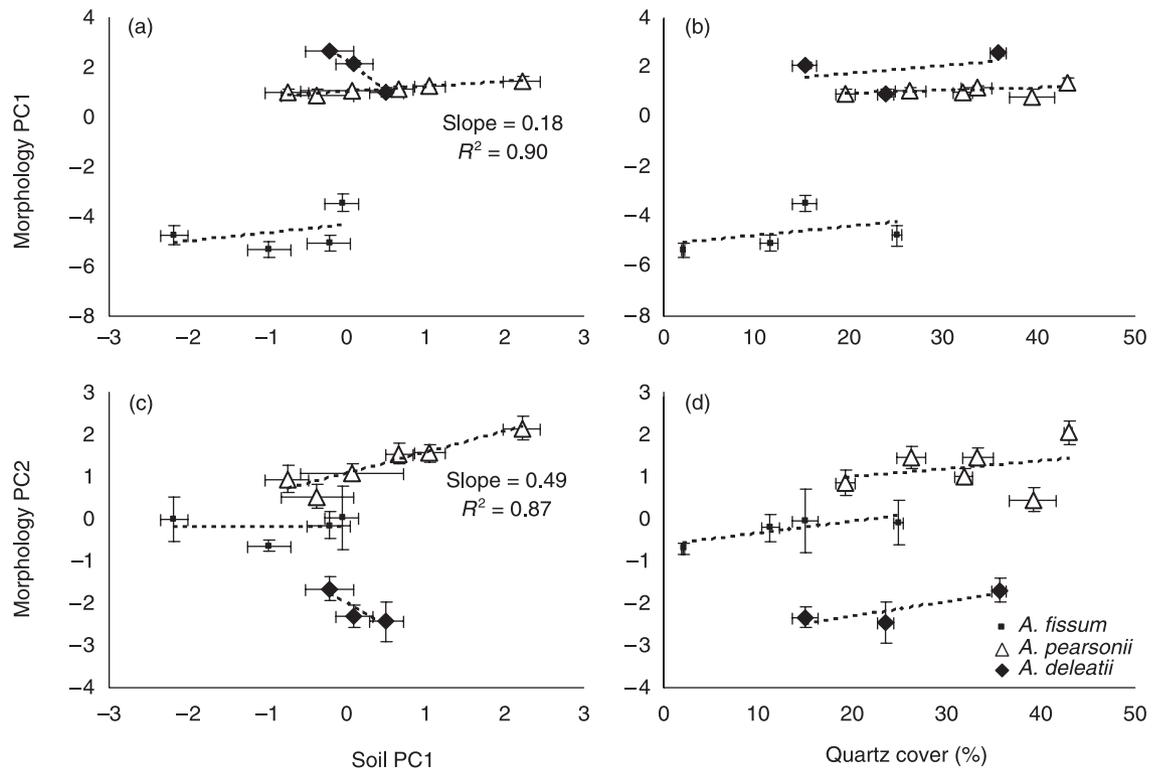
interaction terms were significant in the analyses of pH, stone content and quartz cover, suggesting that habitat use patterns vary between sites (Fig. 4).

The first principal component of a PCA of soil pH, conductivity and stone content accounted for 45.58% of the variance between soil samples. pH loaded most heavily and was negatively correlated with this axis. Both conductivity and stone content contribute positively to PC1. Figure 5 illustrates the relationship between the edaphic variables (quartz cover and the first axis of the soil PCA) and the morphological axes

along which the focal species pairs are most clearly separated (morphology PC1 for *A. fissum* and *A. pearsonii* and morphology PC2 for *A. delaetii* and *A. pearsonii*). The clear morphological separation between *A. fissum* and the specialists on the first morphological axis corresponds to habitat use differences in terms of both quartz cover and soil PC1 (Fig. 5a,b). *A. pearsonii* and *A. delaetii* are clearly separated along the second morphological axis despite complete overlap along soil PC1 (Fig. 5c,d). Soil PC1 scores are significantly correlated with population scores along both morphological



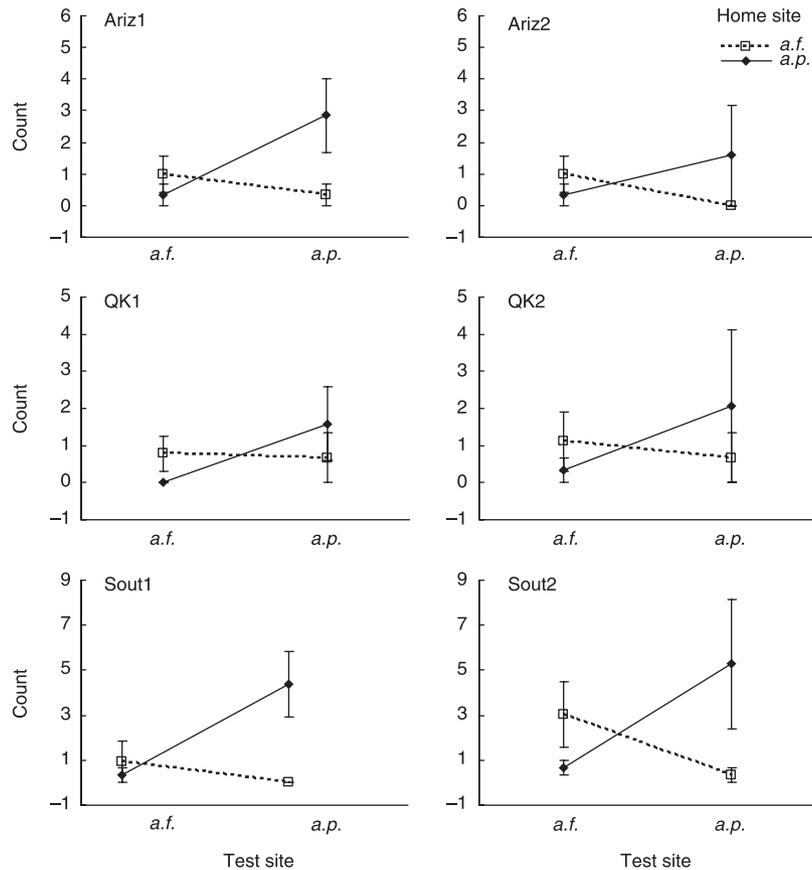
**Fig. 4** Soil characteristics (pH, electrical conductivity, stone content and quartz cover) measured in paired parapatric populations of *A. delaetii* and *A. pearsonii* at three sites. Means and standard errors as well as  $F$ -values for main (species and site) and interaction effects from mixed-model ANOVAs are shown for each variable. No measured soil variables differed significantly between *A. delaetii* and *A. pearsonii* habitats across sites. Asterisks indicate significant a priori contrasts ( $P < 0.05$ ) between species at a given site.



**Fig. 5** Plots illustrating the relationship between divergence in morphology (first and second multivariate PCA axes) and edaphic habitat use (quartz cover and the first soil PCA axis) in the species studied. Points represent population means and error bars are  $\pm 1$  SE. Slopes and  $R^2$  values are given for significant regressions of population means within a species.

**Table 2** Effects of home site (species), test site (environment) and species–environment interaction from two-way ANOVAs comparing mean survival and seedling weight in reciprocal transplant experiments between *A. fissum* (generalist) and *A. pearsonii* (specialist) at six sites where the species occur parapatrically. A significant interaction term indicates local adaptation

Variable	Effect	d.f.	MS	F ratio	P
Number surviving (per 100 seeds)	Species	1	0.22	2.5	0.13
	Environment	1	0.16	1.8	0.20
	Species × environment	1	2.30	25.9	< 0.001
	Error	18	0.09		
Growth rate (of survivors)	Species	1	0.46	12.44	0.002
	Environment	1	0.03	0.73	0.40
	Species × environment	1	0.08	2.08	0.17
	Error	18	0.04		



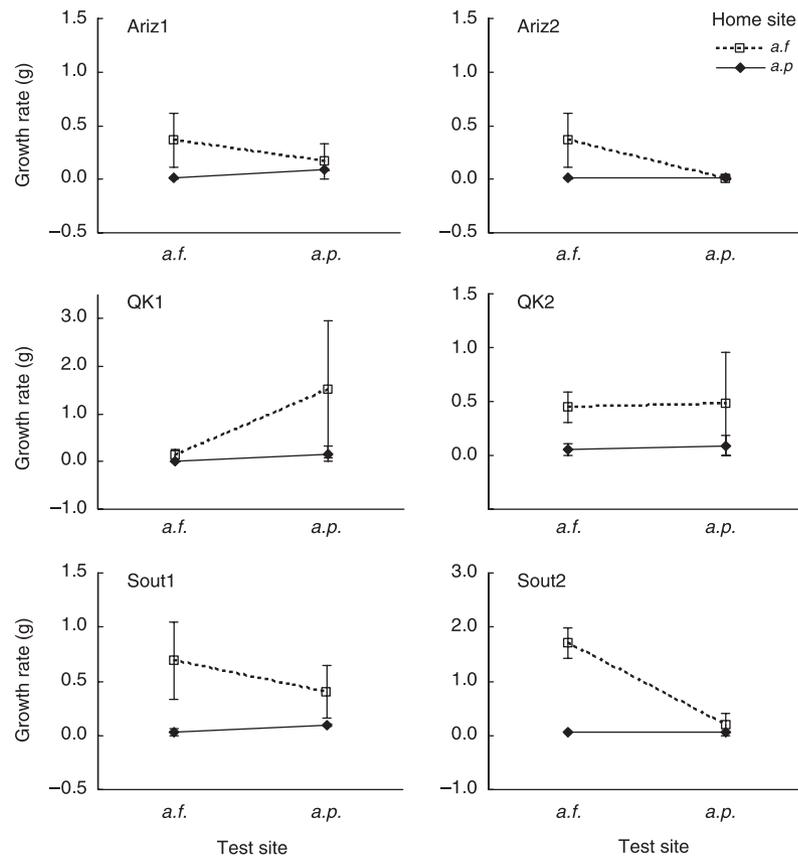
**Fig. 6** Mean survival of seed reciprocally transplanted between *A. fissum* (*a.f.* – generalist) and *A. pearsonii* (*a.p.* – specialist) habitats at six sites where the species occur parapatrically. Lines represent the home site or species being tested and the *x*-axis represents the test site or environment in which the species were tested. Bars represent standard errors around the means for each species-by-environment combination.

axes within *A. pearsonii* (Fig. 5a,c). These intraspecific correlations are in the same direction as the interspecific relationship between morphology and habitat use.

#### TRANSPLANT EXPERIMENTS

Transplant experiments revealed significant home-site advantage in all species tested. In the *A. fissum* × *A. pearsonii* experiment, analysis of survival data revealed a significant home-site × test-site (or species × environment) interaction (Table 2, Fig. 6). Survival was significantly higher in native than in non-native habitats for

both species (*A. fissum*:  $t = 2.65$ , d.f. = 9,  $P = 0.016$ ; *A. pearsonii*:  $t = 4.537$ , d.f. = 9,  $P = 0.0003$ ). There was no main effect of species or environment on survival, but there was a significant effect of species on growth rate of survivors (Table 2, Fig. 7). Survivors of *A. fissum* were significantly heavier than those of *A. pearsonii* ( $t = 3.53$ , d.f. = 20,  $P = 0.002$ ) across environments. At all six sites the measured reaction norms for seed survival in *A. pearsonii* and *A. fissum* cross, indicating a consistent pattern of local adaptation at all contact zones investigated (Fig. 6). The growth rate pattern was more complicated. Reaction norms did not cross,



**Fig. 7** Mean growth of individuals over 3 years after seed was reciprocally transplanted between *A. fissum* (*a.f.* – generalist) and *A. pearsonii* (*a.p.* – specialist) habitats at six sites where the species occur parapatrically. Lines represent the home site or species being tested and the x-axis represents the test site or environment in which the species were tested. Error bars represent standard errors around the means for each species-by-environment combination.

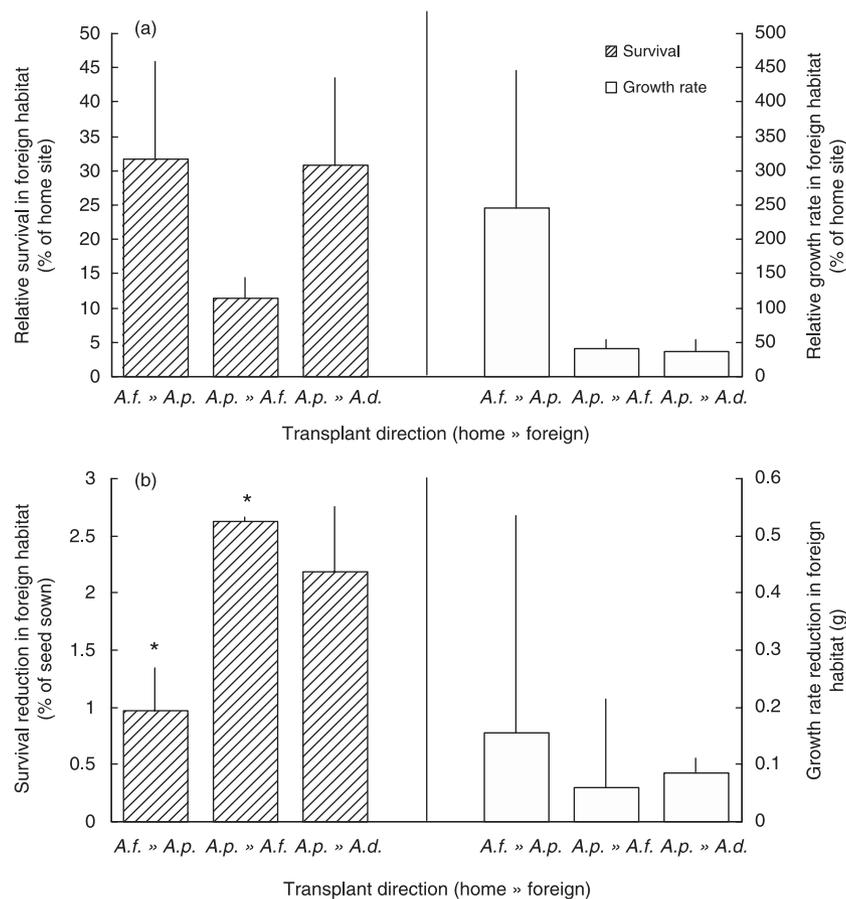
**Table 3** Results of two-way mixed-model ANOVAs investigating the difference between survival and mass of *A. pearsonii* seedlings in native and *A. delaetii* habitat. Environment is the test site (*A. pearsonii* vs. *A. delaetii* habitat) and the random factor, population, is the location where the experiment was performed (four contact zones between *A. pearsonii* and *A. delaetii*). A significant main effect of environment indicates home-site advantage

Variable	Effect	d.f.	MS	F ratio	P
Number surviving (per 100 seeds)	environment	1	1.90	14.11	0.033
	population	3	1.06	7.91	0.062
	environment × population	3	0.13	0.31	0.821
	error	16	0.44		
Growth rate (of survivors)	environment	1	0.29	19.31	0.021
	population	3	0.03	2.01	0.290
	environment × population	3	0.02	0.17	0.914
	error	16	0.09		

and the interaction term was not significant, although at five of the six sites growth rates were higher in native habitat for both species (Table 2, Fig. 7). In the second experiment, *A. pearsonii* showed significant reduction in survival and growth when transplanted into populations of *A. delaetii* (Table 3).

The reduction in survivorship in non-native relative to native sites was between 68% and 88% (Fig. 8a). Whereas the growth rate of the specialist, *A. pearsonii*, was reduced by 60–64% in foreign habitat, the generalist exhibited higher growth rates in *A. pearsonii*

habitat (Fig. 8a). This surprising result was, however, driven by a single *A. fissum* site, which if excluded from the analysis results in a mean growth rate reduction in foreign habitat of 55% across the other sites sampled. The generalist (*A. fissum*) in *A. pearsonii* habitat had threefold higher relative survivorship and sixfold higher relative growth rate than *A. pearsonii* in *A. fissum* habitat. Thus, as expected, the magnitude of the transplant effect is greater for the specialist than for the generalist although this difference was not significant for the relative fitness measures (survival:  $t = 1.25$ , d.f. = 5,



**Fig. 8** (a) Relative survival and growth rate in non-native habitat measured as the percentage of home-site fitness attained in transplanted treatments. (b) Absolute reduction in survival (percentage of seeds sown) and growth (mean mass of survivors) in non-native habitat compared with native habitat. *A.f.* » *A.p.* represents *A. fissum* (generalist) transplanted to *A. pearsonii* (specialist) habitat. *A.p.* » *A.f.* and *A.p.* » *A.d.* represent *A. pearsonii* (specialist) transplanted to *A. fissum* (generalist) and *A. delaetii* (specialist) habitat, respectively. Data are means across replicate sites. Error bars are  $\pm 1$  SE. Asterisks indicate significant differences from paired *t*-tests comparing *A.f.* » *A.p.* and *A.p.* » *A.f.* as well as *A.p.* » *A.f.* and *A.p.* » *A.d.*

$P = 0.267$ ; growth rate:  $t = 0.98$ , d.f. = 5,  $P = 0.375$ ). There was, however, a significant difference between absolute reduction in survival of *A. pearsonii* in *A. fissum* habitat and *A. fissum* in *A. pearsonii* habitat (survival:  $t = 4.33$ , d.f. = 5,  $P = 0.007$ ; growth rate:  $t = 0.98$ , d.f. = 5,  $P = 0.375$ ). Whereas *A. pearsonii* survival was reduced by 2.52% in generalist habitat, *A. fissum* survival was only reduced by 0.98% in specialist habitat (Fig. 8b). There were no significant differences in survival or growth rate of *A. pearsonii* transplanted into the habitat of the generalist vs. the habitat of the other specialist species (*A. delaetii*).

## Discussion

The soils of the quartz fields of the Knersvlakte represent an osmotically challenging environment. In addition to osmotic stress, high salt content can cause ion toxicity, oxidative stress and nutrient deficiency (Gorham 1992; Gueta-Dahan *et al.* 1997). Sodium levels measured in this study are 100 $\times$  higher than those reported for serpentine soils (Rajakaruna & Bohm 1999) and 1000 $\times$  higher than levels used in studies investigat-

ing salinity stress in the *Iris hexagona* system (van Zandt *et al.* 2003). Electrical conductivity (or ionic strength) is 10 $\times$  higher and manganese concentrations, although not high enough to be toxic (Brault *et al.* 1994), are 10 $\times$  higher in Knersvlakte than in serpentine soils. Concentrations of all other elements measured are equivalent to those reported for serpentine soils (Rajakaruna & Bohm 1999). The Knersvlakte edaphic environment thus represents a strong selective regime which is potentially as unique as that posed by serpentine soils, a habitat type which has long served as the model system for studying edaphic endemism in plants (Kruckeberg 1984; Macnair & Gardner 1998). The uniqueness of both selective regimes is further supported by the high levels of floral endemism in both habitat types: 215 serpentine endemic taxa in California alone (Kruckeberg 1984) and 52 quartz-field endemics on the Knersvlakte (Schmiedel & Jurgens 1999).

The endemic quartz-field flora is dominated by unusual growth forms, mostly nanochamaephytes (dwarf shrubs < 5 cm in height) and geophytes (Schmiedel & Jurgens 1999). The dwarf shrubs are invariably succulent and may have compact (e.g. *A. fissum*), subglobose

(e.g. *A. pearsonii*) or subterranean (e.g. *A. delaetii*) growth forms (Fig. 1). The morphological trend between *Argyroderma* species occupying sparse (*A. fissum*) and dense (*A. pearsonii* and *A. delaetii*) quartz habitats is towards reduction in plant size and height (dwarfism), increased levels of submergence and decreased branching (Figs 2 & 5). Dwarfism and reduced branching are traits which characterize quartz-field species generally, across independent lineages and between the six major quartz-field regions in southern Africa (Jurgens 1986; Ihlenfeldt 1994; Schmiedel & Jurgens 1999). The above authors proposed that these growth forms are the result of convergent evolution in response to the strong selective regime associated with the unusual edaphic environments of the quartz fields. In *Argyroderma* we also show a trend towards spherical leaf shapes (decreased area : volume ratio), increased leaf thickness and increased enclosure of the leaf by the old leaf sheaths. These are all traits with a clear functional role in water-loss reduction in osmotically stressed environments (Fearn 1977; Gibson 1996).

The suite of characters which vary between *A. fissum* and the quartz specialists could therefore reflect adaptive morphological optima in dense and sparse quartz environments. Reciprocal transplants of *A. fissum* and *A. pearsonii* lend support to this possibility. Both growth forms had significantly higher survival in their native habitats (Fig. 6). However, local adaptation detected in this experiment reflects differences in survival and growth of seedlings during the first 3 years of life and thus extrapolation of the results to adult morphological traits may not be appropriate. The home-site survival advantage demonstrated here may result from physiological traits which were not measured. Plants are known to be physiologically more sensitive to salinity in early developmental stages (Gorham 1992). However, the data do suggest that *A. pearsonii* has a significantly lower growth rate than *A. fissum* (Table 2, Fig. 7), which is itself thought to represent an adaptation to osmotically stressful environments (Grime 1979; Chapin 1980; Bennington & McGraw 1995). Differences in growth rate between species could result in divergence of many of the adult traits measured (e.g. branch number, plant size). Although not significant, both species tended to exhibit reduced growth rates in non-native environments (Fig. 7, Table 2). Furthermore, preliminary transplants of adults of the three species studied suggests that both survival and reproduction are reduced in non-native habitat (A. G. Ellis, unpublished data).

In addition to exhibiting reduced survival when transplanted into the habitat of the generalist (i.e. sparse quartz cover), *A. pearsonii* also had significantly reduced survivorship and growth when transplanted into the edaphic environment of the other quartz specialist species (Table 3). This suggests that quartz specialists themselves may be locally adapted to specific edaphic environments within the quartz gravel-plain habitat. Schmiedel & Jurgens (1999) suggested that

edaphic environments within the quartz habitat form a continuum between those with lower stone content, higher pH and high salinity and those with high stone content, low salinity and low soil pH. In addition, they showed that subglobose (e.g. *A. pearsonii*) and subterranean (e.g. *A. delaetii*) growth forms vary in the edaphic environments they occupy. In this study we found no overall significant differences between the habitats occupied by *A. pearsonii* and *A. delaetii*, although the subterranean growth form (*A. delaetii*) did occur on habitats with lower quartz cover at all contact zones studied (Fig. 4). These species do, however, exhibit functional morphological differences (submergence, leaf size, sheath persistence and enclosure) and only very rarely occur interspersed on the same edaphic substrate. Further work is required to reveal the components of the edaphic environment which may have led to adaptive divergence between these quartz specialist taxa.

The evidence suggests that coexistence of these three sympatric species is facilitated by local adaptation to edaphic microenvironments. The community structure studied here, consisting of a compact, a subglobose and a subterranean species, is common throughout the range of the genus (Hartmann 1978; A. G. Ellis, personal observation). Edaphic specialization may thus be an important mechanism maintaining the mosaic microspatial distribution patterns within *Argyroderma*. The spatial scale at which these species respond to edaphic selection gradients is remarkably small. Transplant arenas in this study were located between 10 m and 1000 m apart. The evolutionary trend towards dwarfism on the quartz gravel plain habitat may itself have contributed to the fine spatial scale of the evolutionary response to edaphic heterogeneity in *Argyroderma*. Reduced size restricts exposure of individual plants to very small patches of soil and also allows persistence of viable populations on small habitat patches, both factors which would facilitate adaptation to edaphic microhabitats in *Argyroderma*, which has highly localized seed dispersal (Desmet *et al.* 1998). Edaphic specialization may thus be an important factor producing the mosaic microscale distribution patterns which are a general striking feature of quartz-field floras in southern Africa (Schmiedel & Jurgens 1999).

Schmiedel & Jurgens (1999) further suggested that larger quartz-field growth forms (compact nanochamaephytes) have less specialized habitat preferences than smaller forms (subglobose and subterranean). In *Argyroderma*, *A. fissum*, the compact species, has a wide distribution and is thought to have general habitat requirements, whereas the other 10 species are narrowly distributed quartz specialists (Hartmann 1978). To some extent the patterns of local adaptation revealed by transplant experiments support this idea. The specialist had threefold greater home-site survival advantage and sixfold greater home-site growth advantage than the generalist (Fig. 8a). However, this trend was not significant, perhaps as a result of the high

variance in response of the generalist across sites. The specialist did exhibit a significantly higher absolute reduction in survivorship than *A. fissum* (Fig. 8b).

Hartmann (1978) proposed that multiple evolutionary lineages of quartz specialist taxa arose from a generalist ancestor (equivalent to *A. fissum*). She further envisaged an evolutionary trend towards reduction within these specialist lineages (i.e. sparsely branched subglobose to unbranched subterranean forms) in response to environmental gradients. This study suggests that adaptation to edaphic microenvironments may have been instrumental in divergence between species within the genus, in particular between the generalist and the specialists, but also between specialist taxa. The local adaptation we demonstrate probably arises from fitness reduction due to trade-offs associated with distinct trait optima in different environments, and thus rules out the possibility that the morphological differences between species are a result of plastic responses to environmental heterogeneity. Instead, reciprocal transplants suggest a role for divergent selection in the diversification of *Argyroderma*. However, as is the case with all transplant experiments, we are unable to eliminate the alternative possibility that the phenotypes studied (compact, subglobose and subterranean) represent alternative states on a fitness ridge in phenotype-by-environment space and not a series of fitness peaks (Emerson & Arnold 1989; Schluter 2000). Under this scenario, the likelihood that transitions between phenotypes can occur through genetic drift is increased because these transitions do not involve crossing fitness valleys. In order to confirm the presence of adaptive peaks (i.e. an unequivocal role for divergent selection) it would be necessary to show that intermediate phenotypes have reduced fitness in intermediate habitats. The evidence from *Argyroderma* suggests that both the underlying edaphic variation and the phenotypic response to it are not discontinuous, and thus the fitness surface in this system may well be more like a ridge than a series of separate peaks. Nonetheless, the data do suggest an important role for edaphic specialization in the coexistence and diversification of *Argyroderma* in the Knersvlakte and possibly in the remarkable radiation of the Aizoaceae in southern Africa.

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