Evolution, 60(1), 2006, pp. 000–000

EVOLUTIONARY RADIATION OF ''STONE PLANTS'' IN THE GENUS *ARGYRODERMA* (AIZOACEAE): UNRAVELING THE EFFECTS OF LANDSCAPE, HABITAT, AND FLOWERING TIME

ALLAN G. ELLIS,[1,2,3,*] ARTHUR E. WEIS,[1,4] AND BRANDON S. GAUT[1,5]
[1]*Department of Ecology and Evolutionary Biology, 321 Steinhaus Hall, University of California, Irvine, California, 92697*
[3]*E-mail: monkeybeetle@gmail.com*
[4]*E-mail: aeweis@uci.edu*
[5]*E-mail: bgaut@uci.edu*

Abstract.—Recent phylogenetic evidence suggests that the extraordinary diversity of the Cape Floristic Kingdom in South Africa may be the result of widespread evolutionary radiation. Our understanding of the role of adaptive versus neutral processes in these radiations remains largely speculative. In this study we investigated factors involved in the diversification of *Argyroderma*, a genus within the most spectacular of the Cape radiations, that of the Ruschioid subfamily of the Aizoaceae. We used amplified fragment length polymorphisms and a suite of morphological traits to elucidate patterns of differentiation within and between species of *Argyroderma* across the range of the genus. We then used a matrix correlation approach to assess the influence of landscape structure, edaphic gradients, and flowering phenology on phenotypic and neutral genetic divergence in the system. We found evidence for strong spatial genetic isolation at all taxonomic levels. In addition, genetic differentiation occurs along a temporal axis, between sympatric species with divergent flowering times. Morphological differentiation, which previous studies suggest is adaptive, occurs along a habitat axis, between populations occupying different edaphic microenvironments. Morphological differentiation is in turn significantly associated with flowering time shifts. Thus we propose that diversification within *Argyroderma* has occurred through a process of adaptive speciation in allopatry. Spatially isolated populations diverge phenotypically in response to divergent habitat selection, which in turn leads to the evolution of reproductive isolation through divergence of flowering phenologies, perhaps as a correlated response to morphological divergence. Evidence suggests that diversification of the group has proceeded in two phases: the first involving divergence of allopatric taxa on varied microhabitats within a novel habitat type (the quartz gravel plains), and the second involving range expansion of an early flowering phenotype on the most extreme edaphic habitat and subsequent incomplete differentiation of allopatric populations of the early flowering group. These results point to adaptive speciation in allopatry as a likely model for the spectacular diversification of the ice-plant family in the dissected landscapes of the southern African winter rainfall deserts.

Key words.—AFLPs, Aizoaceae, edaphic selection, flowering phenology, South Africa, spatial genetic structure, succulent karoo.

Received April 8, 2005. Accepted September 23, 2005.

Adaptive radiations are characterized by the accumulation of substantial morphological and ecological diversity within rapidly speciating lineages (Schluter 1996, 2000). Evolutionary radiations are adaptive if divergent natural selection stemming from the environment and resource competition plays a dominant role in phenotypic diversification and the buildup of reproductive isolation between taxa (Schluter 2000). Adaptive radiations have undoubtedly played a central role in the origin and proliferation of taxa on oceanic archipelagos (e.g., Galápagos finches, Grant 1986; Hawaiian silverswords, Baldwin and Sanderson 1998; Caribbean *Anolis* lizards, Jackman et al. 1997). However, with the obvious exception of the extraordinary radiations of the cichlid fishes in the East African rift lakes (Meyer 1993; McCune 1997), the role of adaptive radiation in generating the diversity of life on the continental land masses is less clear.

A recently accumulated body of molecular phylogenetic evidence from a wide range of plant families does, however, point toward widespread, recent, rapid radiation as the origin of the exceptional species richness and endemism of the continental Cape Floristic Region in South Africa (Rhamnaceae, Richardson et al. 2001; Iridaceae, Reeves et al. 2001; Goldblatt et al. 2002; Poaceae, Verboom et al. 2003; Geraniaceae, Bakker et al. 1999a,b, Restionaceae, Linder et al. 2003; Aizoaceae, Klak et al. 2004; but see Linder and Hardy 2004). Our understanding of the degree to which the functional, morphological, and species diversity within the Cape lineages is dictated by adaptation to the environment remains largely speculative. Although topographic, climatic, and edaphic diversity have long been considered important drivers of plant speciation in the winter rainfall regions of the Cape (Linder 1985, 2003; Cowling et al. 1992), only Verboom et al. (2004) have directly demonstrated the role of edaphic adaptation in the radiation of one Cape genus, *Ehrharta*.

Most recently, Klak et al. (2004) showed that the highly diverse, arid-adapted Ruschioid subfamily of the Aizoaceae has undergone extensive radiation in the winter-rainfall succulent karoo region of southern Africa. Approximately 1563 species within the Ruschioideae, 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). Remarkably, the radiation of these succulent plants in the southern African deserts is equivalent in both tempo and extent to the radiations reported for cichlid fishes of the East African rift lakes, arguably the most dramatic and fastest examples of living adaptive radiations known (Schluter 2000).

*Corresponding author.
[2] Present address: Department of Biological and Conservation Sciences, University of Kwazulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa.

The remarkable species and growth form diversity within the Ruschioid radiation has been attributed to the interaction between diverse edaphic habitats and both extrinsic and intrinsic restrictions to gene flow (Ihlenfeldt 1994; Klak et al. 2004). The highly specialized hygrochastic fruit capsules of the Ruschioideae, which only release their seed when wet, limit seed dispersal in space and spread it through time by effectively coupling dispersal and germination cues (Hartmann 1988; Parolin 2001). Limited seed dispersal in the highly dissected landscapes of the succulent karoo renders genetic drift a likely mechanism of divergence between populations (Ihlenfeldt 1994). However, no data exist to support or refute this possibility. The role of adaptive response of populations to divergent selection regimes in generating the morphological and, more importantly, the species diversity within the group is equally unknown. This study, which investigates the role of edaphic habitat gradients and landscape structure (geographic isolation) as determinants of the patterns of morphological and neutral genetic differentiation within *Argyroderma*, represents the first attempt to tease apart the roles of selection and genetic drift in evolutionary divergence and, ultimately, speciation, within the Ruschioid radiation (also see Ellis and Weis 2006).

Argyroderma contains 11 taxa (Table 1), all of which are confined to the Knersvlakte region of the succulent karoo, an area of approximately 100 × 80 km in extent (Hartmann 1978). The genus has diversified onto the regionally unique quartz gravel plain habitat that characterizes the Knersvlakte. Ten taxa are quartz specialists, exhibiting reduced, stonelike growth forms associated with the quartz gravel plain habitat to which they are confined (Fig. 1). The eleventh taxon, the generalist, is highly branched and mat forming, growing on both quartz gravels and the surrounding quartz-poor matrix habitat. Reciprocal transplant experiments suggest that divergence in potentially functional morphological traits between *Argyroderma* species is likely the result of adaptation to edaphic microenvironments (Ellis and Weis 2006).

The quartz habitat is confined to erosional basins within the Knersvlakte landscape (Fig. 2). The distributions of *Argyroderma* taxa reflect the strong spatial structure of the habitat they occupy (Hartmann 1978). Each quartz specialist taxon is confined to one or a few quartz-containing erosional basins within the landscape, whereas the generalist is widespread, occurring sympatrically with the majority of specialist populations. The existence of generalist-specialist species pairs in isolated drainage basins within the landscape, together with Hartmann's (1978) suggestion from taxonomic data that the reduced specialist growth forms have arisen on at least two occasions from a ''generalist-like'' ancestor, points to the possibility that the specialist taxa may have arisen on multiple occasions through parallel ecological speciation driven directly by selection across the quartz-nonquartz habitat boundary.

In this study we used amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) and a suite of morphological traits to elucidate patterns of differentiation within and between species of *Argyroderma* across the geographic range of the genus. We then used a matrix correlation approach to assess the influence of landscape structure, edaphic gradients, and flowering phenology on phenotypic and neutral genetic divergence in the system. Through this approach we aimed: (1) to investigate the extent and scale of spatial genetic isolation in the system, (2) to evaluate the influence of divergent selection imposed by edaphic factors on differentiation, and, ultimately (3) to propose a mechanistic hypothesis for the evolutionary diversification of the group.

Materials and Methods

Study System

The landscape

Argyroderma N. E. Br. (Aizoaceae) is confined to the Knersvlakte region of the Western Cape Province of South Africa (30°45′ to 31°40′ S, 18°15′ to 19°00′ E). The Knersvlakte area (about 100 × 80 km) comprises a highly eroded, deflating drainage basin, consisting of a number of smaller erosion units associated with individual river systems (Fig. 2). Removal of fine particles (silts and clays) from the soil profile by water runoff has resulted in the formation of extensive quartz gravel plains on the landscape surface, each associated with an individual drainage system. The quartz gravels are most continuous within the central and eastern drainage basins (GeelU, GeelL, and Sout basins), with isolated patches of quartz habitat also found in basins to the west (Rooi, Moed, and Koek) and south (Troe) of this area (Fig. 2). The quartz habitat is not homogenous, varying in both the characteristics of the quartz itself (e.g., pebble density, pebble size, pebble composition) and of the soil matrix (e.g., pH, salinity, soil depth). Schmiedel and Jurgens (1999) describe two distinct quartz-field edaphic habitats that represent the extremes of a habitat continuum and support separate plant communities. On one extreme are 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. The edaphic heterogeneity of the area results from the diversity of quartz-intruded shales, phyllites, and limestones of the Precambrian Nama group sediments that underlie it (De Beer et al. 2002).

The plants

Argyroderma is a genus of compact, perennial dwarf succulents that falls within the highly diverse arid-adapted subfamily, the Ruschioideae, in the family Aizoaceae (Bittrich and Hartmann 1988). Hartmann (1978) delimited 10 species in the genus *Argyroderma*, one of which has two subspecies (see Table 1 for names and Fig. 1 for illustrations). Ten taxa, the quartz specialists, have highly reduced growth forms and are confined to the quartz gravel plain habitat. The eleventh taxon, the generalist, is widespread, exists on both quartz and quartz poor habitats, is highly branched, and has long fingerlike leaves.

The specialist taxa can be divided into two major groups on the basis of morphology and flowering time, which Hartman (1978) interprets as representing at least two independent evolutionary lineages toward quartz specialization from a generalistlike ancestor. The first group, which we refer to as the delaetii group, consists of four largely allopatric species (Table 1) that are often unbranched and usually grow sunken

TABLE 1. Genetic diversity indices, geographical information, Bolus herbarium voucher numbers (Bol no.) and labeling of all populations sampled for amplified fragment length polymorphism analysis of *Argyroderma*. Morphological group, species name, population name, drainage basin, latitude (decimal degrees), longitude, number of individuals sampled (*n*), number of bands per population (loci), percentage of polymorphic bands (P%), Nei's gene diversity (H_e) and number of rare bands per individual ± SE (rare) are reported for each population sampled.

				Geography			Genetic diversity				
Group	Species	Pop. ID	Bol. no.	Basin	Latitude	Longitude	*n*	Loci	P%	H_e	Rare
Delaetii group	*A. congregatum*	co_GM	Ellis 03	Rooi	−31.30767	18.46208	5	181	39.1	0.1787	3.2 ± 0.7
		co_Koek2	Ellis 02	Koek	−31.45917	18.31333	5	174	40.3	0.1914	1.6 ± 0.5
		co_Moed	Ellis 01	Moed	−31.44436	18.43356	5	185	42.7	0.1953	3.0 ± 0.5
	A. crateriforme	cr_Ariz	Ellis 08	GeelL	−31.33314	18.66775	5	197	44.7	0.2011	1.6 ± 0.5
		cr_DTG	Ellis 06	Sout	−31.41436	18.68933	5	182	39.5	0.1780	1.4 ± 0.5
		cr_DTG2	Ellis 05	Sout	−31.42200	18.68664	5	192	42.3	0.1878	3.4 ± 0.9
		cr_GGrd	Ellis 04	Rooi	−31.28092	18.51353	5	191	46.2	0.2041	2.2 ± 0.2
		cr_Kbg	Ellis 07	GeelU	−31.16778	18.56083	5	179	35.2	0.1732	3.8 ± 0.8
		cr_KBG2	—	GeelU	−31.20222	18.49972	4	170	33.6	0.1708	4.3 ± 0.9
		cr_Vdp	—	Troe	−31.62786	18.72936	5	181	38.3	0.1739	3.0 ± 0.4
	A. ringens	ri_Vbg1	Ellis 24	Troe	−31.52250	18.91833	5	172	36.4	0.1701	2.2 ± 0.6
		ri_Vbg2	—	Troe	−31.53167	18.92333	5	179	39.1	0.1740	3.0 ± 0.4
	A. delaetii	de_QK	Ellis 09	Sout	−31.41478	18.64492	5	202	49.0	0.2145	4.6 ± 0.8
		dl_Ariz2	Ellis 11	GeelL	−31.34158	18.66925	5	192	42.3	0.1911	5.0 ± 0.5
		dl_GGt	Ellis 10	GeelU	−31.26531	18.54803	5	195	44.7	0.2013	3.4 ± 0.2
Framesii group	*A. patens*	pa_FV1	Ellis 21	GeelU	−31.28328	18.58511	5	200	46.6	0.2096	3.4 ± 1.2
		pa_FV2		GeelU	−31.27861	18.58722	4	196	44.3	0.2154	5.3 ± 0.8
	A. pearsonii	pe_S1	—	Sout	−31.38533	18.68478	5	180	36.0	0.1673	4.2 ± 0.6
		pe_S2	—	Sout	−31.38894	18.67606	5	186	40.7	0.1806	1.8 ± 0.6
		pe_S3	Ellis 22	Sout	−31.41683	18.64264	5	180	37.9	0.1695	1.6 ± 0.2
		pe_S4	Ellis 23	Sout	−31.41389	18.65472	5	197	45.8	0.1983	2.6 ± 0.5
		pe_S5	—	GeelL	−31.34086	18.67544	5	188	43.9	0.1999	2.4 ± 0.7
		pe_S6	—	GeelL	−31.34397	18.67383	5	189	43.9	0.1952	2.4 ± 0.7
	A. testiculare	te_Ariz3	Ellis 27	GeelL	−31.32219	18.65689	5	173	32.8	0.1549	4.2 ± 0.4
		te_Ariz4	Ellis 28	GeelL	−31.32186	18.66311	5	190	39.1	0.1736	4.0 ± 0.6
	A. framesii ssp. *hallii*	fh_GGrd	Ellis 19	Rooi	−31.35394	18.46375	5	189	45.8	0.2109	4.2 ± 0.9
		fh_Moed	Ellis 20	Moed	−31.44481	18.43389	4	171	33.2	0.1699	7.5 ± 0.6
	A. subalbum	su_Koek	Ellis 25	Koek	−31.47825	18.30106	4	165	24.9	0.1416	10.3 ± 0.3
		su_Koek2	Ellis 26	Koek	−31.45917	18.31333	4	157	19.4	0.1222	11.3 ± 1.0
	A. framesii ssp. *framesii*	ff_FV1	Ellis 18	GeelU	−31.28328	18.58511	5	182	36.8	0.1663	5.4 ± 1.0
		ff_GGt	Ellis 17	GeelU	−31.26528	18.54789	5	178	35.6	0.1702	4.2 ± 0.7
Generalist	*A. fissum*	f_FV	—	GeelU	−31.28639	18.58250	5	168	30.0	0.1458	4.0 ± 0.7
		f_GM	—	Rooi	−31.30767	18.46208	3	154	22.5	0.1499	2.0 ± 0.6
		f_Moed	Ellis 16	Moed	−31.44514	18.43278	5	167	32.8	0.1673	2.4 ± 0.5
		f_S1	Ellis 14	Sout	−31.38517	18.68569	6	176	40.3	0.1699	2.8 ± 0.4
		f_S2	Ellis 15	Sout	−31.38894	18.67606	5	161	32.4	0.1584	1.0 ± 0.3
		f_S3	Ellis 12	Sout	−31.41583	18.64244	5	172	34.8	0.1568	2.2 ± 0.7
		f_S4	Ellis 13	Sout	−31.41447	18.65169	4	175	30.4	0.1638	2.3 ± 0.3

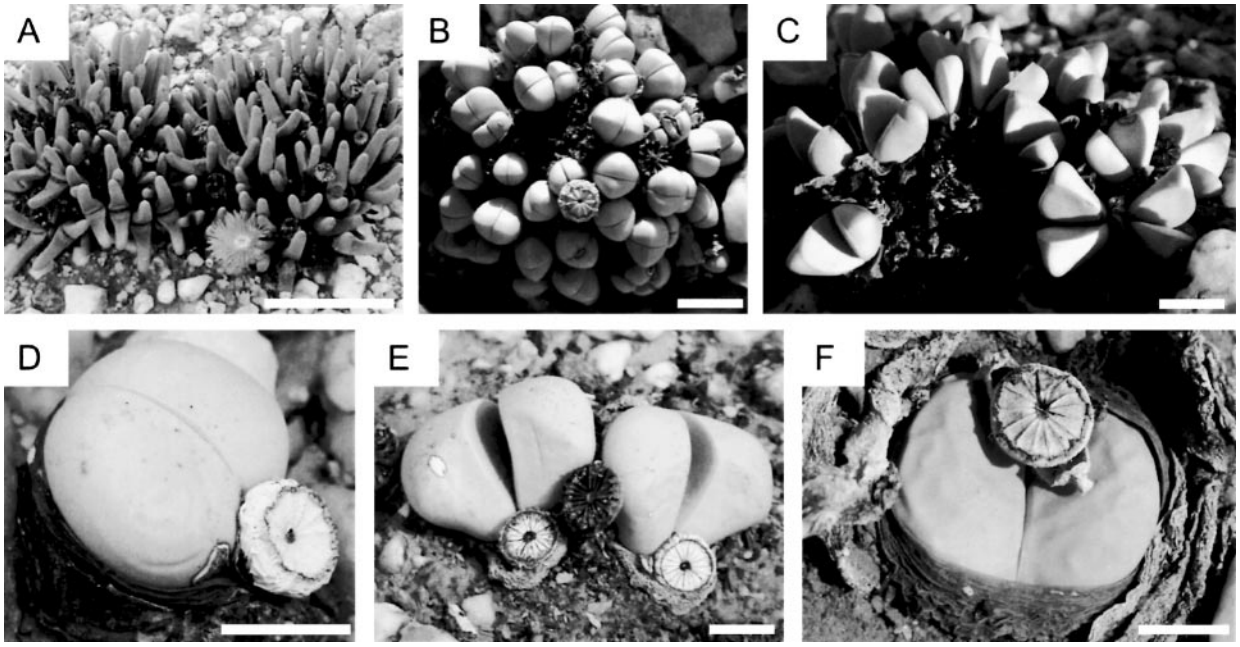

FIG. 1. The range of morphological variation within *Argyroderma*. The generalist (A), three framesii-group species (B–D) and two delaetii-group species (E–F) are illustrated. (A) *A. fissum*, the generalist, illustrating the matlike growth form and long fingerlike leaves. (B) *A. framesii* sp. *framesii*; highly branched with small subglobose leaves. (C) *A. patens*; branched with angular, spreading leaves. (D) *A. pearsonii*; unbranched with large subglobose leaves. (E) *A. crateriforme*; large hood shaped, spreading leaves. (F) *A. delaetii*; unbranched and submerged. Scale bars are 1 cm except in *A. fissum* where the scale bar represents 10 cm. Photos by A. G. Ellis.

into the soil surface. Shared traits include a low concentration of idioblasts in the leaves (resulting in old leaves that are yellow and nonpersistent) and fruits with more than 12 locules and that are not strongly persistent on the parent plant. The group flowers early, from April to May, although a few *A. delaetii* populations flower in June. The second group, the framesii group, contains six largely allopatric taxa (Table 1) that are morphologically more diverse than the delaetii-group taxa. They are branched (except *A. pearsonii*) and have a persistent leaf sheath that is red (except in *A. testiculare*) as a result of high leaf idioblast concentrations. The group flowers during the second part of the flowering season (May–August) and fruits have 12 or fewer locules and are persistent on the parent plant. Populations of the two morphological groups often coexist in sympatry (or in close parapatry). Framesii-group populations always occur sympatrically with delaetii-group populations, whereas delaetii-group populations may also occur in isolation.

Argyroderma plants produce numerous seeds in loculicidal, hygrochastic capsules (Klak et al. 2004). The seed lacks inherent dormancy (Hartmann 1978) and thus primary seed dispersal distances are likely to be short, associated with rainfall runoff immediately following seed release during rainfall events. *Argyroderma* species are obligately outcrossing and flowers are cuplike, pollinated primarily by small solitary bees (Struck 1995; A. G. Ellis, unpubl. data). Although floral morphology does not vary much within the genus, the color of the petaloid staminodes does. Flowers of the generalist and framesii-group are predominantly magenta, whereas delaetii-group species usually have white, yellow, or pink flowers. Flower color often varies between populations within a species and sometimes even within a population (Hartmann 1978). All species with overlapping flowering times can be crossed, producing hybrid seed that is no less viable than seed from intraspecific crosses (Hartmann 1978; Hammer and Liede 1990). Although rare, hybrid individuals and even hybrid populations are known from the field (Hartmann 1978). All species are diploid and have the same number of chromosomes ($2n = 18$; Hartmann 1978). Reproductive isolation in the group appears to be achieved largely through separation of flowering phenologies (Hartmann 1978). Species flower in a predictable sequence that is maintained under greenhouse conditions, suggesting that flowering time is to some extent genetically controlled.

Sampling Strategy

Between 1999 and 2001 we sampled the full taxonomic and geographic range of *Argyroderma*. We aimed first, to sample multiple populations of all recognized species; second, to sample representative species and populations from multiple drainage basin units; and third, to sample sympatric generalist-specialist population pairs from several drainage basins. Morphological data were collected from five to 15 individuals from each of 39 populations, and five individuals were sampled from each of 37 populations for genetic analysis. We characterized the edaphic habitat occupied by each population and collected flowering phenology data in 30 populations for two years. Representative herbarium specimens were deposited in the Bolus herbarium (BOL), in the Botany Department at the University of Cape Town, South Africa (see Table 1 for voucher numbers). In addition, the individuals sampled for genetic analysis were deposited in the live collection of S. Hammer, Bolus Herbarium.

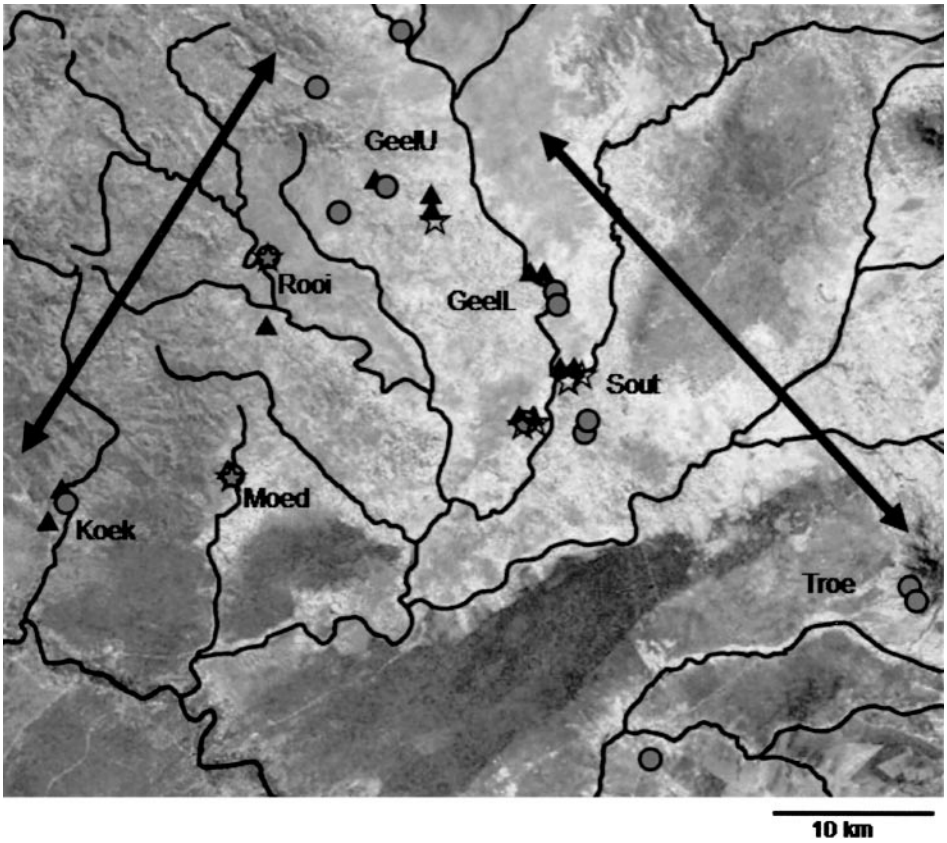

FIG. 2. Satellite image of the Knersvlakte region illustrating the structure of the drainage system. Names refer to individual drainage basins containing the quartz pebble plain habitat, which is visible as the white areas on the map. Quartz-rich sediments only exist in drainage basins to the southwest of a fault striking southeast to northwest roughly corresponding to the current course of the Geelbeks river (GeelU and GeelL). White areas to the east and southeast of this fault are exposed shales and do not contain quartz. Symbols indicate the spatial arrangement of sampled populations (circles, delaetii group; triangles, framesii group; stars, generalist). The two arrows represent the geographic axes of genetic differentiation in the delaetii group (see Fig. 4).

Genetic Structure

Because of the low levels of sequence divergence reported across the 1563 species in the Ruschioideae (Klak et al. 2004), we used AFLP markers (Vos et al. 1995), which are more likely to provide higher numbers of variable markers than sequence data, to elucidate patterns of genetic differentiation in the system. DNA was extracted from wet leaf material (100 mg) using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA). We followed the AFLP plant mapping kit protocol (PE Applied Biosystems, Foster city, CA) with only minor modifications, which will be described briefly. Restriction (MseI and EcoRI) and ligation reactions were performed simultaneously at room temperature overnight. Polymerase chain reaction (PCR) amplification was performed in two steps using 10-μl reaction volumes following Vos et al. (1995). We used a subsample of eight individuals to screen 40 selective primer pairs from which we selected three (E.TG/M.CTT, E.AA/M.CAC, E.AC/M.CAT) that produced clear, reproducible bands and displayed variation between and within populations. To assess repeatability of the data we replicated all reactions, including DNA extraction, for a subset of 15 individuals and found that bands were 100% reproducible in all cases. Fluorescently labeled selective PCR products were separated on 5% denaturing Long Ranger gels (Bio Whittaker Molecular Applications, Rockland, ME) using an ABI Prism 377 DNA Sequencer with Genescan-500-ROX (PE Applied Biosystems) as an internal lane size standard. Raw data were extracted using Genescan Analysis software (PE Applied Biosystems). Potential AFLP loci were initially located automatically with Genotyper software (PE Applied Biosystems) by searching for peaks higher than 500 florescence units within the 50–500 bp size range, 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 greater than 0.2 bp or if peak heights varied substantially across individuals. Results were exported as a binary presence/absence matrix for subsequent analyses.

Gene diversity and genetic differentiation parameters were estimated assuming that comigrating fragments represent homologous loci and that populations are at Hardy-Weinberg equilibrium. We used the methods of Lynch and Milligan (1994) as implemented in AFLPsurv (Vekemans et al. 2002) to estimate population allele frequencies to calculate the percentage of polymorphic loci (P%) and Nei's (1978) unbiased gene diversity (H_e). In addition, the number of private alleles (bands confined to a single population) and the number of rare alleles per individual (bands present in less than 10% of all individuals) were estimated for each population.

Genetic divergence between populations was estimated using the unbiased estimator of Nei's (1972) genetic distance (Lynch and Milligan 1994) implemented in AFLPsurv (Vekemans et al. 2002). PHYLIP (Felsenstein 1985) was used to construct a neighbor-joining tree from the matrix of genetic distances. Statistical support of nodes was assessed using 1000 resampled matrices generated by bootstrapping across loci in AFLPsurv. Analysis of molecular variance (AMOVA), as implemented in Arlequin (Excoffier et al. 1992), was used to investigate the hierarchical structuring of genetic variation in the system, both with regard to landscape structure and on the basis of the existing taxonomic divisions within the genus. Total genetic variance was partitioned in two ways: (1) taxonomically: among species, among populations within species, and within populations; and (2) geographically: between drainage basins, among populations within drainage basins, and within populations. All hierarchically partitioned AMOVAs were conducted on multiple partially nested datasets (i.e., all populations, specialist populations only, framesii-group populations only, and delaetii-group populations only). This approach allowed assessment of genetic patterns at various levels in the dataset; in other words, between the generalist and specialist groups, between specialist taxa, and between the largely allopatric populations within the delaetii and framesii groups. In addition, estimates of population differentiation (Φ_{ST}) from AMOVA were calculated at each level of analysis.

Genetic structure was further elucidated by using the model-based Bayesian clustering methods implemented in the program STRUCTURE (Pritchard et al. 2000) to objectively identify the number of genetic clusters (K) present within the multilocus AFLP dataset and to assign individuals to identified clusters. We used an admixture model, which allows individuals to have mixed ancestry, and assumed that population allele frequencies were correlated. We used burn-in and data collection periods of 100,000 iterations each for runs spanning $K = 1$–16. For $K > 10$ the clustering algorithm found multiple solutions (with distinct posterior probabilities), a situation that was not improved by increasing the length of the runs. To overcome this we ran 10 simulations for each $K > 10$ and chose the solution with the highest mean posterior probability across runs for each K. Individual assignment coefficients were used to determine the proportion of membership of each population in each of the genetic clusters identified.

Morphological Differentiation

To characterize patterns of phenotypic differentiation between populations we measured 21 morphological traits for five to 15 adult individuals in each of 39 populations. The traits measured were the same as those used by Hartmann (1978) in her taxonomic treatment of the genus. Seven whole plant characteristics were measured: mean plant diameter, height of the plant above the soil surface, proportion of the leaf below the soil surface, plant shape (width/length), number of branches, fruit production, and the number of old capsules retained. Eight leaf characteristics were measured on three leaf pairs per individual, where possible, and character averages were calculated for each individual. Leaf traits measured were: maximum leaf pair diameter, leaf length, leaf thickness, leaf pair shape, leaf volume, length of the gap between leaves in a pair, proportion of the leaf contained within the sheath, and the number of leaf sheaths retained. Six 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, capsule shape, number of locules, length of the fruit bract, distance between the bract and the capsule rim, and proportion of fruit enclosed within the leaf sheath.

For the purposes of this paper we used standardized population trait means to compute Euclidean distances between populations in multivariate morphological space, which served as an index of morphological differentiation between populations. Principal components analysis (PCA; SAS Institute 2001) was used to visualize the clustering of individuals in multivariate morphological space. A total of 266 individuals was clustered on the basis of 18 normally distributed morphological characters. Percentage data were arcsine-transformed for analysis. Plant diameter, plant height, leaf number, leaf pair length, leaf thickness, leaf volume, and leaf height data were positively skewed and heteroscedastic and were thus log-transformed before analysis.

Landscape Structure, Soil Characteristics, and Flowering Time

Landscape structure

We calculated the geographic distance between all population pairs from GPS coordinates using ArcGIS (Environmental Sciences Research Institute, Redlands, CA). In addition, we assigned populations to one of seven drainage units (Koek, Moed, Rooi, GeelU, GeelL, Sout, and Troe; Fig. 2), each encompassing a separate river system, with the exception of GeelU and GeelL, which represent the disjunct upper and lower reaches of the Geelbeks River. Drainage basin assignments were then used to generate a binary pairwise population dissimilarity matrix (0, same drainage unit; 1, different drainage unit) representing landscape structure.

Flowering time

The flowering phenology of 30 populations was investigated during 2000 and 2001. All flowering individuals were counted weekly in 10–30 m^2 plots within each population for the duration of the four-month flowering period. A matrix of differences in peak flowering week (averaged between years) was constructed to represent the degree of temporal separation between populations.

Soil characteristics

Soil pH, electrical conductivity, and stone content were measured within each population in order to characterize edaphic conditions. These variables were used because they have been shown by Schmiedel and Jurgens (1999) to be important determinants of plant community composition on the Knersvlakte. Three bulked soil samples, each consisting of five 1000 cm^3 soil cores, were collected from central and peripheral locations within each population. Soil was passed

TABLE 2. The spatial distribution of genetic diversity indices within the morphological species groups. Percentage of polymorphic bands (P%) and rare alleles are reported as the means ± SE across populations within species groups within drainage basins. Drainage basins are arranged along an arc from southeast to southwest.

	P%			Rare alleles		
Basin	Delaetii group	Framesii group	Generalist	Delaetii group	Framesii group	Generalist
Troe	37.9 ± 0.8	—	—	2.7 ± 0.3	—	—
Sout	43.6 ± 2.8	40.1 ± 2.1	34.5 ± 2.1	3.1 ± 0.9	2.6 ± 0.6	2.1 ± 0.4
GeelL	43.5 ± 1.2	39.9 ± 2.6	—	3.3 ± 1.7	3.3 ± 0.5	—
GeelU	37.8 ± 3.5	40.8 ± 2.7	30	3.8 ± 0.3	4.6 ± 0.5	4
Rooi	42.7 ± 3.5	45.8	22.5	2.7 ± 0.5	4.2	2
Moed	42.7	33.2	32.8	3	7.5	2.4
Koek	40.3	22.2 ± 2.8	—	1.6	10.8 ± 0.5	—

through a 1-mm sieve and the stone content was calculated by weight. Electrical conductivity (mS/cm) was measured through a soil paste, and pH was determined in a 0.1M KCl solution at Matrolab, Cape Town, South Africa. Edaphic differentiation was calculated as the distance between population means along the first principal component of a PCA of the measured edaphic variables.

Matrix Correlation

We used a multiple regression extension of the Mantel test of matrix correspondence (Smouse et al. 1986; Legendre et al. 1994) to assess the contribution of landscape structure, geographical distance, soil habitat, and flowering time to both genetic and morphological divergence within the system. Similar partial matrix correspondence tests (PMCTs) have been widely used to investigate the influence of independent factors best represented by distance matrices (in particular geographic distance) on phenotypic and genetic differentiation between populations (e.g., Thorpe and Malhotra 1996; Ogden and Thorpe 2002). Because elements within the distance matrices being compared are not independent, the significance of the observed regression parameters (R^2 and partial regression coefficients) was determined by randomly permuting the dependent matrix. We used a backward elimination procedure with 10,000 permutations to select significant predictor variables. Analyses were conducted using the program PERMUTE! (Legendre et al. 1994). Matrices of Nei's (1978) unbiased genetic distance and of Euclidean distances (based on 21 morphological traits) between populations served as the response variables in two separate sets of multiple regression analyses. The matrices of geographical distance, differences in drainage basin occupancy, edaphic differentiation, and divergence in peak flowering time between populations served as the independent variables in both multiple regression models. In addition, the genetic distance matrix was included as an independent variable in the analysis of factors contributing to morphological divergence in the system.

Analyses were conducted using an iterative process involving a series of partially nested regression models, which allowed us to investigate factors contributing to divergence at various taxonomic levels within the dataset. The most inclusive level used (i.e., all populations) allowed assessment of factors associated with the divergence between the generalist and specialist groups. Next we analyzed all specialist populations, framesii-group populations, and delaetii-group populations separately. We then eliminated the isolated western populations from the analysis of the framesii-group species in order to explore factors associated with divergence of populations in the main body of the quartz field habitat. The final analysis explored factors associated with intraspecific divergence within *A. pearsonii*, the species for which we had sampled the most populations ($N = 6$). Significance levels of R^2 and partial regression coefficients were adjusted accordingly, using Bonferroni correction.

RESULTS

Genetic Structure

The final binary genetic matrix consisted of 253 AFLP loci scored across 183 individuals. All individuals had unique AFLP profiles with an average of 129.8 (SE 0.5) fragments per profile. Estimates of the percentage of polymorphic bands in *Argyroderma* populations range from 19.4 to 49.0% (Table 1). Estimates of Nei's (1978) gene diversity within populations ranged from 0.1222 to 0.2154. Only a single private allele was detected, which was confined to the *A. framesii hallii* population in the isolated Moed basin in the west (Fig. 2). Estimates of the mean number of rare fragments per individual in each population ranged from 1.0 to 11.3 (Table 1). The delaetii group shows no obvious spatial structure in the distribution of population genetic diversity indices (Table 2). Framesii-group populations from the western basins (Moed and Koek) have low polymorphism and high numbers of rare alleles relative to delaetii-group populations and the framesii-group populations from the central basins (Table 2). Generalist populations have low levels of polymorphism (Tables 1 and 2).

The neighbor-joining phenogram, constructed from a matrix of Nei's (1972) genetic distance between populations, revealed a well-supported basal split between the generalist and specialist populations (Fig. 3). Within the specialist group, all populations, except the framesii-group populations from the two isolated western basins (Koek and Moed), form a well-supported and largely unresolved cluster. Within the specialist polytomy, only populations of the framesii-group species *A. pearsonii*, *A. testiculare*, and *A. framesii framesii* form species-level clusters with reasonable bootstrap support. Populations within the delaetii group do not group into species, although there is some statistical support for a cluster of delaetii-group populations (*A. crateriforme* and *A. ringens*) from the southern basins (Troe and Sout). Within the gen-

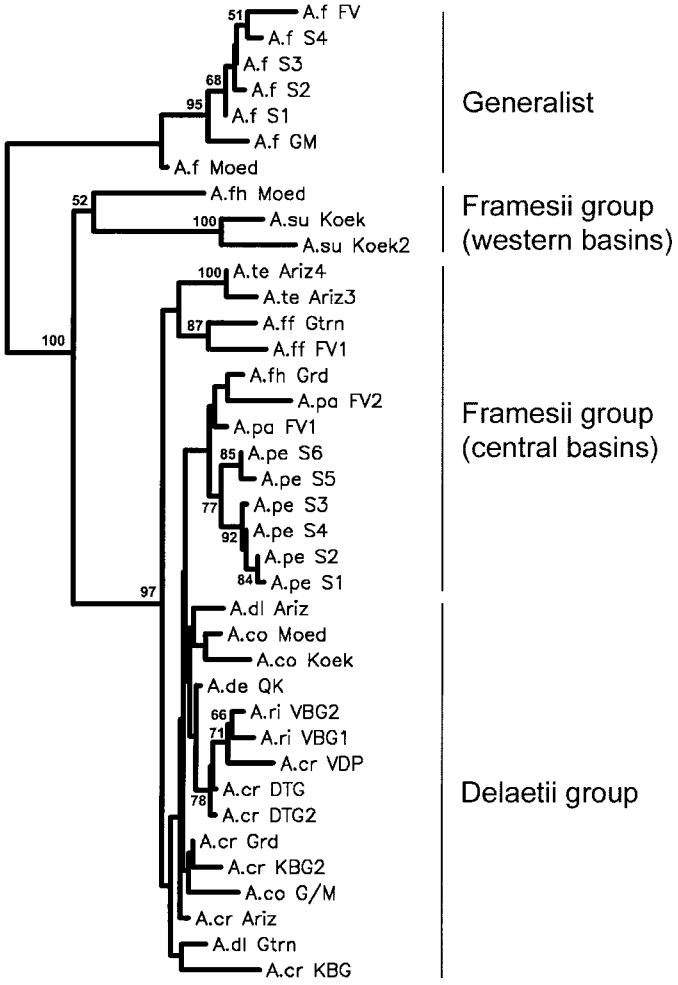

FIG. 3. Neighbor-joining tree constructed from a matrix of Nei's (1972) genetic distance between populations (see Table 1 for species names). Bootstrap values greater than 50% are indicated. AFLPsurv (Vekemans et al. 2002) was used to calculate genetic distances and to generate bootstrap matrices whereas PHYLIP (Felsenstein 1985) was used for neighbor-joining analysis and to estimate support of nodes.

eralist group, populations from the central basins form a well-supported cluster separated from the *A. fissum* population sampled in the western Moed basin (Fig. 3).

Hierarchical AMOVAs, grouping populations by species or by drainage basin, reveal significant taxonomic and geographic structure at all levels in the AFLP dataset (Table 3). When considering all populations, a large component of genetic variance occurs among species (25.37%), but only 4.21% of variance occurs among drainage basins whereas 30.81% of variance occurs between populations within basins (i.e., between sympatric species within drainage basins). This pattern is maintained when considering specialist populations only. Among specialist populations, 13.6% of genetic variance occurs between species whereas 6.69% occurs between basins and 18.91% occurs between sympatric framesii and delaetii-group populations within basins (Table 3). Within the framesii-group 20.14% of genetic variance occurs between species whereas 13.96% occurs between drainage basins. Among-group variance was considerably lower in the delaetii group (3.79% between species, 7.05% between basins) where a larger proportion of the variance occurred within populations (83%). The global estimate of population differentiation from AMOVA (Φ_{ST}), which is analogous to F_{ST}, was $\Phi_{ST} = 0.25$ ($P < 0.001$) for all populations. Specialist populations ($\Phi_{ST} = 0.25$, $P < 0.001$) were more strongly differentiated than generalist populations ($\Phi_{ST} = 0.17$, $P < 0.001$). Similarly, populations within the framesii group ($\Phi_{ST} = 0.29$, $P < 0.001$) were more strongly differentiated than those within the delaetii group ($\Phi_{ST} = 0.16$, $P < 0.001$). Population differentiation within a single range-restricted framesii-group species (*A. pearsonii*) was low ($\Phi_{ST} = 0.07$, $P < 0.001$).

Bayesian clustering analysis of the full dataset yielded a most probable estimate of $K = 14$ genetic clusters in the dataset. All individuals of the generalist, *A. fissum*, formed a distinct genetic cluster with very low levels of admixture with other clusters in the dataset (data not shown). In separate analyses excluding generalist individuals, $K = 13$ clusters were identified within the specialist group with highest posterior probability (Table 4). Six of these clusters grouped individuals within the framesii group and seven grouped individuals within the delaetii group. The genetic clusters within the framesii group largely correspond to species identity (Fig. 4A). The only exception is the cluster grouping *A. patens* populations with the *A. framesii hallii* population from the Rooi basin. Very little admixture was detected between the framesii-group clusters and populations generally have high (>80%) membership proportions in a single cluster (Fig. 4A).

The seven genetic clusters inferred for the delaetii group do not correspond to species identities (Fig. 4B), but instead are structured geographically. The delaetii-group genetic clusters are spatially arranged along two geographical axes, one running southeast to northwest across the quartz fields in the central and eastern parts of the Knersvlakte, and the other running northeast to southwest along the western and northern extremes of the Knersvlakte (Fig. 2). Whereas limited admixture occurs between these two axes, some populations along both axes show high levels of admixture (Fig. 4B), both as a result of comprising individuals from multiple genotypic clusters and containing individuals that are themselves admixed (data not shown). The southeast to northwest axis contains three genetic clusters: the first comprises populations of both *A. crateriforme* and *A. ringens* from the isolated south eastern basin (Troe), the second comprises early-flowering *A. crateriforme* and *A. delaetii* populations from the central basins (Sout and GeelL) and the third comprises late-flowering *A. delaetii* populations from the central basins (Fig. 4B). Admixture occurs predominantly between the early- and late-flowering groups in the central basins. The northeast-southwest axis comprises four populations that correspond to distinct genotypic clusters and two admixed populations that comprise mixtures of genotypes from neighboring populations. All delaetii-group populations have a low proportion of membership (<15%) in framesii-group genetic clusters (Fig. 4B), whereas framesii-group populations with intermediate flowering times (*A. pearsonii* and *A. patens*) have higher membership proportions in delaetii-group genotypic clusters (22–36%; Fig. 4A).

TABLE 3. Hierarchical AMOVAs indicating the distribution of genetic variance among groups (either species or drainage basins), among populations, and within populations. Results of analyses at multiple levels within the dataset are shown: all populations, specialist populations only, and the framesii-and delaetii-group populations only. df, degrees of freedom; SS, sum of squares; VC, variance component; % total, percentage of total genetic variance accounted for at each hierarchical level.

	Species				Basins			
Levels of analysis	df	SS	VC	% total	df	SS	VC	% total
All								
Among groups	10	1974.3	9.58	25.37**	6	704.6	1.56	4.21*
Populations within groups	27	1183.0	4.10	10.85**	31	2452.7	11.42	30.81**
Within populations	145	3491.6	24.08	63.78**	145	3461.6	24.08	64.97**
Specialists								
Among groups	9	995.8	4.57	13.60**	6	608.9	2.24	6.69**
Populations within groups	21	945.8	4.15	12.35**	24	1332.6	6.32	18.91**
Within populations	119	2961.0	24.88	74.05**	119	2961.0	24.88	74.40**
Framesii group								
Among groups	5	633.1	7.12	20.14**	4	493.2	4.88	13.96**
Populations within groups	10	437.6	4.10	11.60**	11	577.5	5.93	16.97**
Within populations	60	1447.5	24.13	68.26**	60	1447.5	24.13	69.07**
Delaetii group								
Among groups	3	199.3	1.18	3.79**	5	338.8	2.18	7.05**
Populations within groups	11	508.3	4.18	13.47**	9	368.7	3.11	10.06**
Within populations	59	1513.5	25.65	82.73**	59	1513.5	25.65	82.90**

** $P < 0.01$, * $P < 0.05$.

Morphological Differentiation

No overlap occurs between the generalist and specialist taxa on the first two principal component axes of the PCA of morphological traits (Fig. 5). The specialist taxa form two largely nonoverlapping clusters, corresponding to the delaetii and framesii groups (Fig. 5). Species in the delaetii group overlap considerably, although a pattern of continuous morphological variation from *A. delaetii* through *A. congregatum* is evident on the first two axes of the PCA (Fig. 5). A similar continuum of variation exists in the framesii group from *A. patens* through *A. framesii framesii*, although *A. patens* is clearly separated along the third principal component. *Argyroderma pearsonii* and *A. testiculare* form a separate cluster within the framesii group that falls intermediately between the delaetii group and the rest of the framesii group and overlaps with them both. The first three axes of the PCA account for 34.5%, 28%, and 7.7% of the variation in the dataset, respectively. Factors loading heavily on the first component are predominantly plant size traits (average plant diameter, plant height, and plant submergence), although two fruit traits (distance from rim of cap to the bracts and the degree to which the cap is enclosed by the withering leaf sheath) load heavily on the first component too. All traits load positively except submergence and capsule enclosure. Leaf size (leaf length, leaf volume, and leaf pair diameter) and fruit size (capsule diameter, locule number, and bract length) traits load most heavily and negatively on the second component.

TABLE 4. Posterior probabilities of the number of genetic clusters (K) inferred in the specialist group using the Bayesian-model-based clustering algorithms implemented in the program STRUCTURE (Pritchard et al. 2000). $P(X/K)$ is the probability of obtaining the observed data, X (the genotypes of the sampled individuals), given they were drawn from K groups. Algorithms identified $K = 13$ clusters with highest posterior probability.

K	ln $P(X/K)$	$P(K/X)$
10	−13,607	~0
11	−13,447	~0
12	−13,448	~0
13	−13,379	~1
14	−13,615	~0
15	−13,453	~0
16	−13,472	~0

Flowering Time

The seasonal pattern of flowering schedules within the genus is represented in Figure 6. Populations flower for an average of 5.5 ± 2.2 weeks during the 17-week flowering period of the genus between mid-April and early August. Populations exhibited a clear seasonal sequence of flowering schedules and differed significantly in terms of peak flowering date for both years (2000: $F_{24,226} = 243.03$, $P < 0.05$; 2001: $F_{24,214} = 288.31$, $P < 0.05$). Populations can be divided broadly into two entirely nonoverlapping flowering time groups (Fig. 6), although extensive overlap in flowering schedule occurs within each of these groups. The early-flowering group contains only members of the delaetii group. The late-flowering group is more diverse, consisting of late-flowering *A. delaetii* populations, the generalist and the majority of the framesii-group species (*A. framesii* ssp. *framesii*, *A. framesii* ssp. *hallii*, *A. subalbum*, and *A. testiculare*). *Argyroderma patens* and *A. pearsonii* exhibit some overlap in flowering schedule with both the late- and early-flowering groups.

Soil Characteristics

Soil analyses distinguish two groups of specialist *Argyroderma* populations that use different edaphic habitats, defined in terms of soil pH and soil stone (quartz) content (Fig.

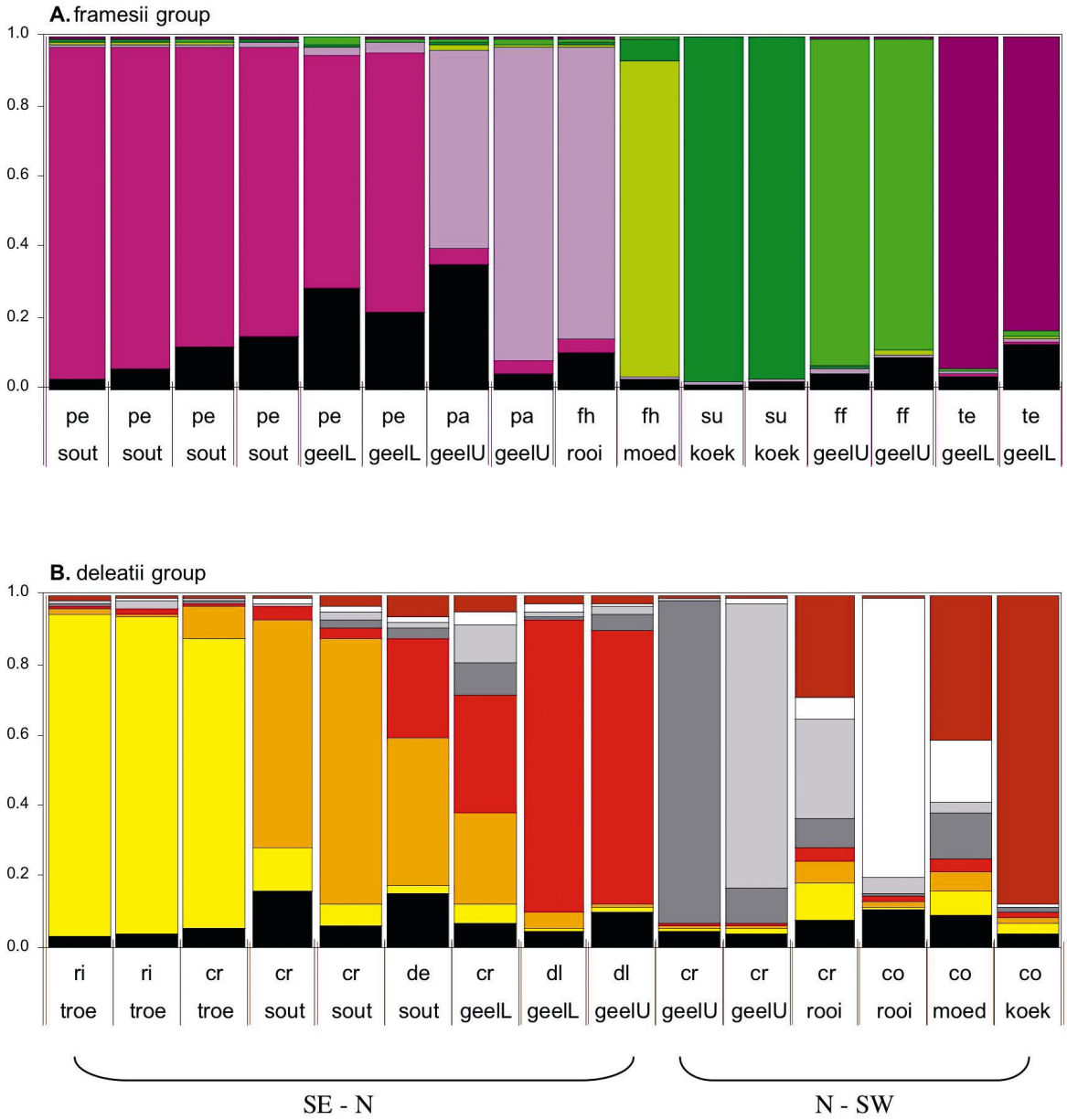

FIG. 4. The proportion of membership of each population in the 13 genetic clusters (each indicated by a separate color) identified from the AFLP dataset by the program STRUCTURE. Populations are identified by species name (see Table 1) and the drainage basin they occupy (see Fig. 2). Framesii-group and delaetii-group clusters are independent except for the black segments which represent the cumulative overlap in membership between the framesii-group and delaetii-group clusters. (A) Membership of the framesii-group populations in the six genetic clusters identified for this group. Genetic clusters correspond to species identity. (B) Membership of the delaetii-group populations in the seven genetic clusters identified for this group. Populations are arranged along two geographic axes (see Fig. 2); one from the southeastern basin (troe) to the northern basin (geelU; yellow, orange, and red clusters) and the second from the northern basin (geelU) to the western basin (koek; gray through brown clusters).

7). The first group uses soils with acidic pH and high stone content, whereas the second group grows on soils with lower stone content and neutral pH. The conductivity (salinity) of the neutral group soils was generally higher than those of acidic group soils. The acidic group comprises the majority of framesii-group species (all populations of *A. patens*, *A. framesii framesii*, *A. framesii hallii*, *A. subalbum*) and only three delaetii-group populations (*A. ringens* and one *A. crateriforme* population). The generalist species grows on neutral group soils as well as on soils with very low stone content (Fig. 7). Soil pH and stone content loaded most heavily on the first principal component of a PCA analysis, which accounted for 55% of the variance in the measured soil variables. We used interpopulation distances along this axis as a measure of divergence in habitat use between populations.

Matrix Correlation

Multiple regressions of geographic distance, drainage basin occupancy, soil difference, and flowering time divergence

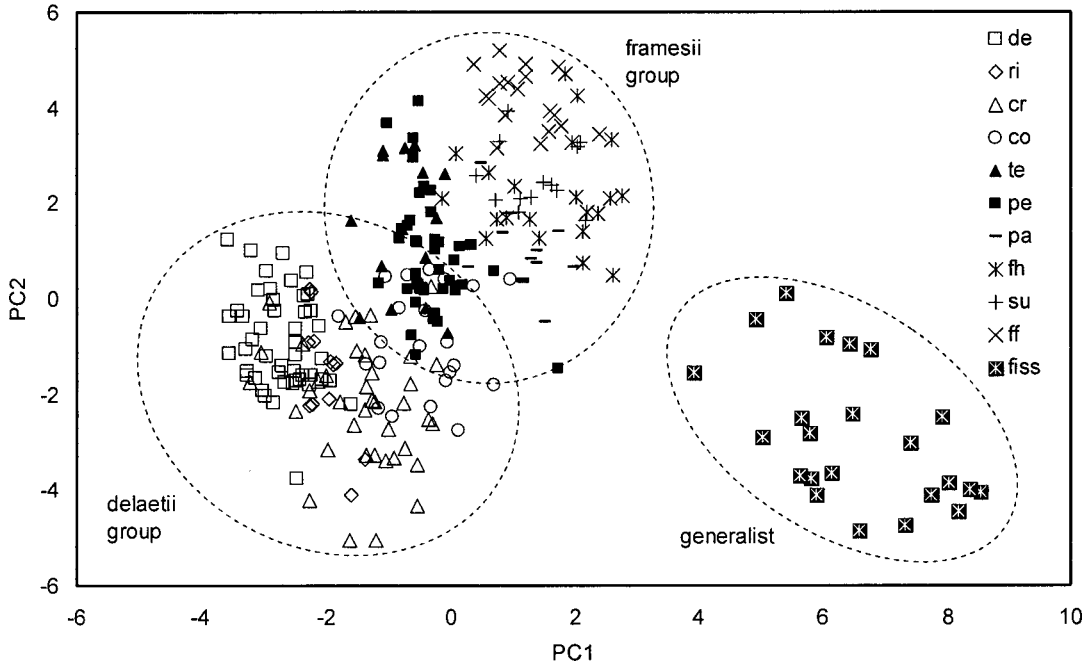

FIG. 5. Plot of the first two axes from a principal components analysis of 18 morphological traits. Symbols represent species (see Table 1 for species names), and each point represents an individual. The framesii-group, delaetii-group, and generalist clusters are encircled. The first two axes account for 34.5% and 28% of the variance in the dataset, respectively. PC1 primarily reflects plant size (average plant diameter and plant height) and growth form (branching and plant submergence). Generalist individuals are large, highly branched, and not submerged. PC2 primarily reflects leaf size (leaf length, leaf volume, and leaf pair diameter) and fruit size (capsule diameter, number of locules, and bract length). Framesii-group individuals have small leaves with lower water storage potential and small fruits with 12 or fewer locules.

on genetic and morphological divergence between populations were significant at all levels of analysis (Table 5). In summary, these analyses showed that genetic divergence between specialist populations is associated with spatial and temporal (flowering time) separation, whereas morphological divergence is associated with habitat differences and flowering time divergence.

Genetic distance between populations within the specialist group did not account for a significant portion of the variance in morphological distance between populations (Table 5), suggesting that morphological divergence does not reflect neutral processes. The significant genetic-morphology correlation in the whole dataset reflects the basal split between the generalist and the specialists. At all levels within the specialist group (i.e., populations within a species through to populations of all species) we found a significant association between geographic variables (either distance or drainage-basin occupancy) and genetic divergence between populations, suggesting that neutral genetic divergence occurs between populations in allopatry. In contrast, morphological divergence is not associated with geographic isolation (Table 5).

Soil habitat differences accounted for a significant component of variance in morphological divergence between specialist populations, in particular within the framesii group, whereas no significant association between soil and genetic divergence was detected in the framesii group (Table 5). However, we did find a significant association between soil and genetic divergence when considering all populations and the specialist populations only, reflecting divergence in habitat use between the genetically distinct generalist and specialist groups and neutral genetic divergence between specialist populations occupying the acidic and neutral habitat types.

Flowering time, or temporal divergence, was associated with genetic divergence in both the framesii and delaetii groups (Table 5), suggesting that neutral genetic differences accumulate between sympatric populations with divergent phenologies. In addition, we found a significant association between morphology and flowering time that is independent of habitat divergence (i.e., soil and flowering time are not correlated; data not shown).

DISCUSSION

The Taxonomic, Spatial, and Temporal Structure of Genetic Differentiation in Argyroderma

The survey of AFLP variation reported here contradicts Hartmann's (1978) proposal that quartz specialists within *Argyroderma* have evolved on multiple occasions from a generalist-like ancestor through parallel speciation. Instead, the 10 recognized specialist taxa form a well-supported cluster which may have diverged basally from the generalist species (Fig. 3), although the alternative possibility, that the generalist was derived from within the specialist group, cannot be excluded. All analyses of the AFLP dataset suggest a predominant influence of landscape structure on genetic divergence between taxa and populations within the specialist cluster (Figs. 3, 4; Tables 2, 3, 5). Genetic divergence occurs between allopatric populations occupying isolated patches of

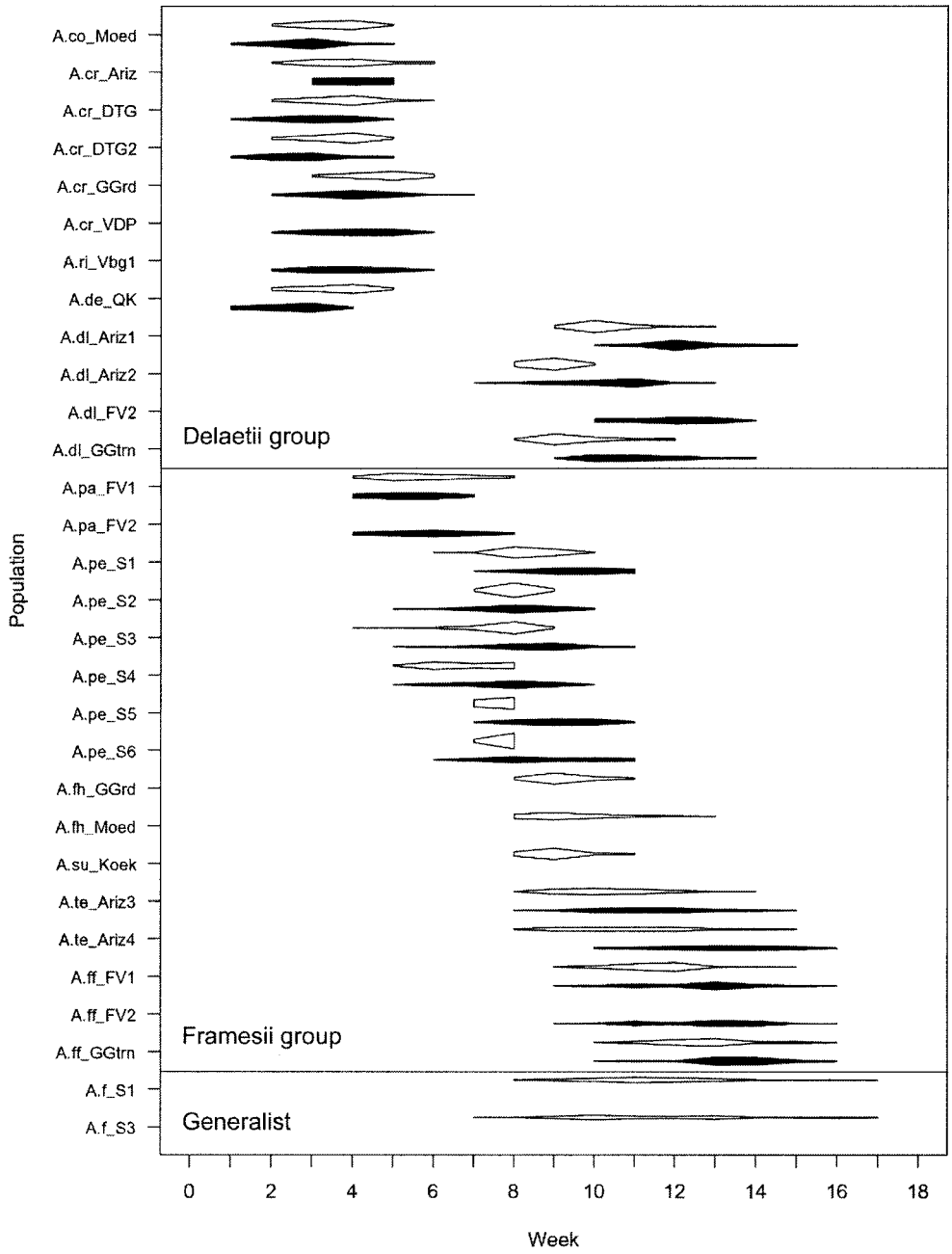

FIG. 6. Plot of population flowering schedules illustrating substantial divergence in flowering phenologies within the genus. See Table 1 for species names. The thickness of polygons represents the proportion of individuals flowering. Open polygons (white) indicate flowering schedules during 2000, and closed polygons (black) represent schedules during 2001. Week 0 begins on 10 April and week 18 ends on the 14 August.

quartz gravel plain habitat associated with the distinct drainage basin units which make up the Knersvlakte landscape. We found evidence for genetic divergence between drainage basins at multiple taxonomic levels: between populations of a single species occupying different drainage basins (Table 5), between populations of the delaetii group irrespective of species identity (Fig. 4; Tables 3, 5) and between allopatric framesii-group taxa (Figs. 3, 4; Tables 3, 5). These data indicate a history of restricted gene flow between drainage basins, a conclusion that is consistent with the results of an intraspecific population genetic survey of the specialist taxon, *A. pearsonii* (A. G. Ellis, unpubl. data).

Whereas the generalist and specialists form two discrete neighbor-joining clusters, the two specialist morphological species groups identified by Hartmann (1978) do not (Fig. 3). We did, however, find that the framesii and delaetii groups comprise distinct, largely nonoverlapping genotypic clusters (Fig. 4). In addition, the framesii and delaetii groups exhibit quite different patterns of genetic differentiation, suggesting independent and distinct evolutionary histories. Neighbor-

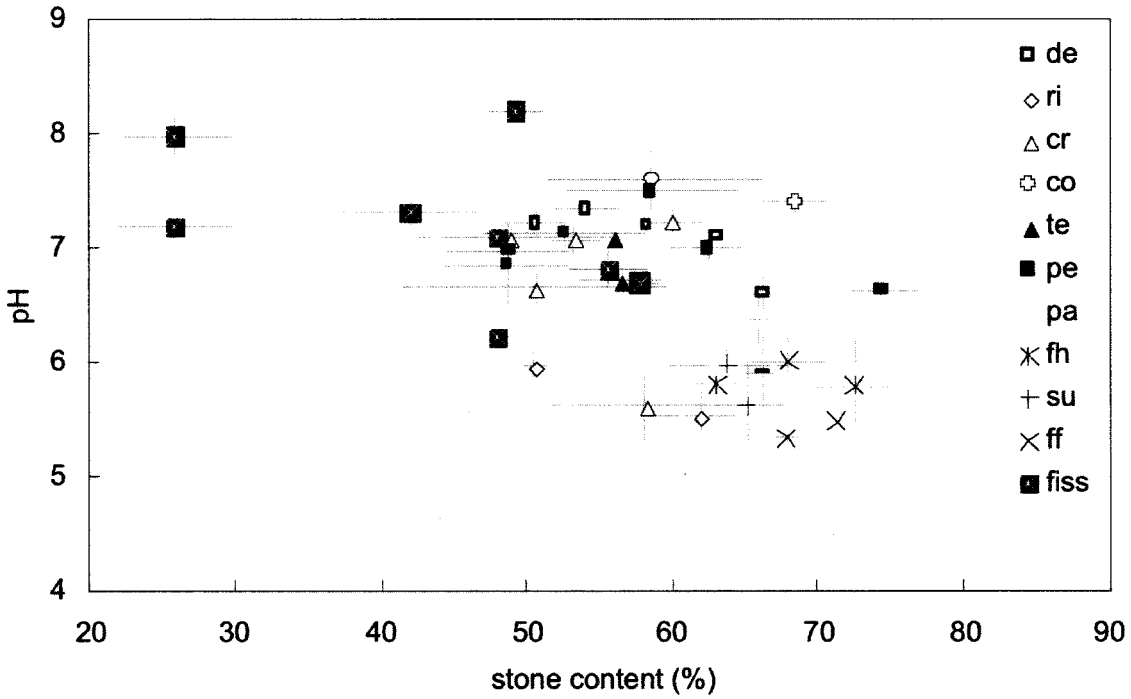

FIG. 7. Plot of soil pH against soil stone content indicating divergence in habitat use between the generalist (fiss; see Table 1 for species names) and the specialists as well as differentiation in habitat use within the specialist group. Specialist populations form two main clusters, one using acidic, high stone content habitats (mostly pa, fh, su, and ff populations) and the other using neutral pH, intermediate stone content habitats. Symbols represent species, points represent population means, and bars represent standard errors.

joining (Fig. 3), AMOVA (Table 3), and Bayesian clustering analyses (Fig. 4) suggested that framesii-group species are strongly differentiated and comprise genetically coherent groups of populations. Delaetii-group populations, on the other hand, do not group into species (Fig. 3), but instead tend to form genetic clusters which correspond to drainage basin occupancy (Fig. 4). In addition, levels of population differentiation in the framesii group are higher than in the delaetii group (Φ_{ST} = 0.29 vs. 0.16) and levels of admixture between framesii-group genetic clusters (i.e., species) are low, whereas substantial spatially structured admixture occurs between delaetii-group genetic clusters (Fig. 4). Finally, the strongly differentiated framesii-group populations from the isolated western basins (Moed and Koek) have reduced gene diversity and high numbers of rare alleles, perhaps suggesting a history of reduced gene flow and strong genetic drift (e.g., Tribsch et al. 2002) although other alternative explanations cannot be eliminated. In contrast the western

TABLE 5. Results of the partial matrix correspondence tests assessing the association between independent distance matrices (indicated in the first row) and both genetic and morphological distance between populations. Results are shown for models including all populations, specialist populations, framesii-group populations, framesii-group populations excluding the western basins (Moed and Koek), delaetii-group populations and *A. pearsonii* populations only. R^2 values for each multiple regression model are indicated. Only significant ($P < 0.05$) partial regression coefficients are indicated (** significant after strict Bonferroni adjustment, * significant after sequential Bonferroni adjustment).

Levels of analysis	*n*	Genetic distance	Geographic distance	Drainage basin	Soil (habitat)	Flowering time	R^2
Genetic distance							
all	27	—			0.2459*		0.0605*
specialists	25	—	0.4459*		0.2618*		0.3058*
framesii group	15	—	0.7713**				0.5949**
framesii group (excl. west)	13	—		0.2446*		0.6268**	0.4539**
delaetii group	10	—	0.6875**			0.4365*	0.5954**
A. pearsonii	6	—		0.7343*			0.5391*
Morphological distance							
all	27	0.6704**				0.2181**	0.5215**
specialists	25				0.3556**	0.3943**	0.2884**
framesii group	15				0.5565**	0.4543**	0.4842**
framesii group (excl. west)	13				0.5682**	0.4623**	0.5469**
delaetii group	10						0.0756 ns
A. pearsonii	6				0.6154*		0.3787*

delaetii-group populations are not strongly differentiated, have higher gene diversity and low numbers of rare alleles. Taken together, this evidence suggests either higher levels of interbasin (and interspecies) gene flow in the delaetii group or that delaetii-group populations result from more recent colonization events or both. Perhaps the most probable explanation is that framesii-group populations have a longer history of isolation, resulting in the strongly differentiated allopatric species which comprise the group. The delaetii group likely emerged from within the framesii group and underwent more recent range expansion, resulting in spatially structured population genetic differentiation, but not well-resolved species. Additional phylogenetic work is required to confirm this hypothesis.

In addition to the clear pattern of genetic divergence between spatially isolated populations, we found evidence for further differentiation associated with temporal or flowering-time divergence between populations in both the framesii and delaetii groups (Table 5). *Argyroderma* species exhibit considerable divergence in flowering phenologies (Fig. 6). This is always true for sympatric specialist species, usually a framesii-group and a delaetii-group species. Thus, temporal divergence likely contributes to the maintenance of genetic distinctiveness between the framesii and delaetii groups. Indeed, we found the highest levels of admixture between framesii- and delaetii-group genotypic clusters in *A. pearsonii* and *A. patens* populations that overlap in flowering time with sympatric early-flowering delaetii-group populations (Fig. 4). In addition, the only sympatric framesii-group species (*A. patens* and *A. framesii framesii*) have divergent flowering times and form distinct genetic clusters (Fig. 4). Similarly, the late-flowering delaetii-group populations comprise a discrete genetic cluster, although admixture does occur into early-flowering sympatric delaetii-group populations (Fig. 4).

The Influence of Edaphic Factors on Differentiation in Argyroderma

Argyroderma species use different edaphic environments (Fig. 7). Each drainage basin contains a fine spatial mosaic of diverse quartz-patch edaphic microenvironments that are used by different *Argyroderma* species (Ellis and Weis 2006). Thus, sympatric species (usually the generalist, a framesii-group species, and a delaetii-group species) are possibly better described as occurring in close parapatry. All specialist species grow on soils with high stone content and dense quartz pebble cover, whereas generalist populations also are found on relatively quartz-free edaphic habitats. The principal axis of divergence in habitat use within the specialist group occurs between framesii-group populations occupying acidic, high-stone-content quartz habitat and delaetii-group and framesii-group species occupying quartz patches with neutral pH, lower stone content and higher salinity (Fig. 7). This is in agreement with Schmiedel and Jurgens (1999), who describe distinct plant communities associated with these two habitat types. These broad divisions in habitat use within *Argyroderma* (i.e., between the generalist and specialists and between specialists occupying acidic and neutral quartz patches) are significantly associated with genetic distance between populations (Table 5), reflecting the coexistence of genetically distinct species using divergent habitats within drainage basins.

In addition, we found that morphological divergence between specialist populations is significantly associated with edaphic habitat differences (Table 5). In contrast, morphological divergence is not associated with AFLP divergence, landscape structure, or geographic distance. This suggests that phenotypic divergence between populations does not occur through neutral stochastic processes, but rather exhibits a signal consistent with an adaptive response to divergent selection imposed by variable edaphic environments. Reciprocal transplant experiments between the generalist, a framesii-group species, and a delaetii-group species showed that species are locally adapted to the edaphic microhabitats they occupy, suggesting that divergence in potentially functional morphological traits probably results from local adaptation (Ellis and Weis 2006). Adaptive responses to unusual edaphic conditions have been demonstrated for many plant taxa (e.g., McNeilly and Antonovics 1968; Fritsche and Kaltz 2000; van Zandt et al. 2003; for further examples see Linhart and Grant 1996).

The morphological trend between *Argyroderma* species occupying sparse (the generalist) and dense quartz habitats (the specialists) is toward reduction in plant size and height (dwarfism), decreased branching, and increased levels of submergence (Fig. 5). The trend between species occupying acidic (*A. framesii*, *A. subalbum*, *A. patens*) and neutral pH quartz-patch environments involves further reduction in size and a tendency towards cessation of branching and largely subterranean growth forms. In addition, species on neutral quartz habitats have substantially larger leaves, which are thicker, able to store higher volumes of water, and tend to be enclosed within the old leaf sheaths (Fig. 5). Interestingly, we found that morphological differentiation between populations is significantly associated with divergence in flowering phenology in the specialist group (Table 5). Branched species with smaller leaf volumes tend to have later flowering times. Similar correlations between flowering time and vegetative morphology have been demonstrated for other plant groups (e.g., Soliva and Widmer 1999).

Mechanisms of Evolutionary Divergence in Argyroderma

We demonstrate a significant influence of landscape structure on genetic divergence in *Argyroderma*, which suggests that gene flow between allopatric populations in isolated drainage basins is restricted (Fig. 8.). Phenotypic divergence, in contrast, is associated with edaphic habitat differences (Table 5). A parallel study suggests that phenotypic differences arise through local adaptation driven by divergent habitat selection (Ellis and Weis 2006). In addition, reciprocal transplants between populations of *A. pearsonii* (A. G. Ellis, unpubl. data) provide evidence for adaptive divergence between basins, but not between habitats within basins, suggesting that local adaptation is facilitated by spatially restricted gene flow in allopatry. However, the evolution of reproductive isolation (i.e., speciation) is not an inevitable outcome of phenotypic differentiation in response to selection (Schluter 2000). In *Argyroderma*, we found that flowering time divergence is associated with morphological dif-

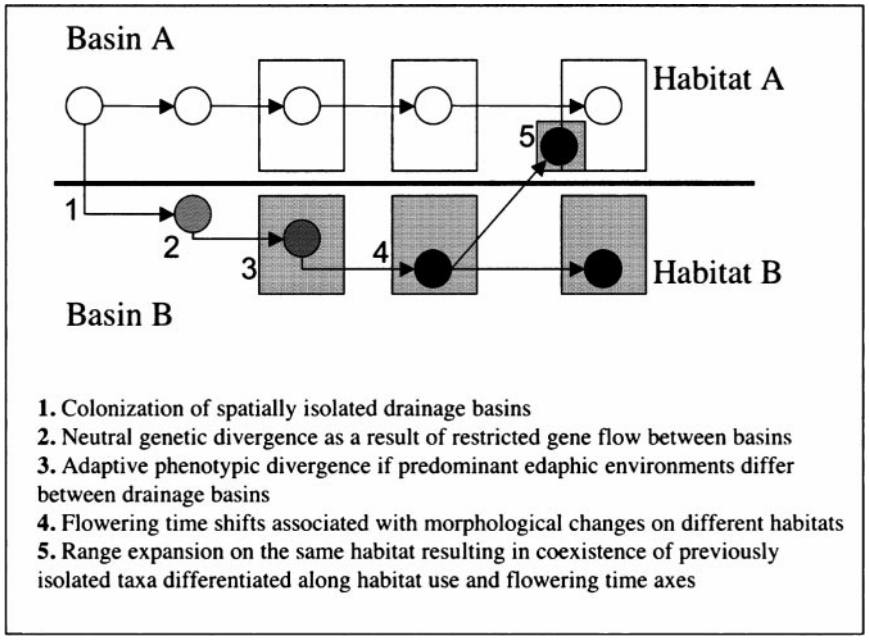

FIG. 8. Proposed model of the evolutionary diversification of *Argyroderma*.

ferentiation (Table 5), which provides a mechanism whereby temporal reproductive isolation between populations may evolve as a result of divergence of morphologies in response to habitat selection. Flowering time shifts may prevent the loss of accumulated phenotypic differences upon secondary contact between previously spatially isolated populations (Fig. 8), and indeed, the patterns of genetic differentiation in *Argyroderma* do suggest that gene flow is restricted between sympatric species with divergent flowering times (Fig. 4). A similar mechanism, involving phenotypic divergence in response to divergent edaphic selection and associated flowering time divergence, perhaps as a pleiotropic by-product, has been proposed for species occupying heavy metal contaminated mine tailings (McNeilly and Antonovics 1968) and for serpentine endemics (Macnair and Christie 1983; Macnair and Gardner 1998).

Thus, we propose that evolutionary diversification of *Argyroderma* has occurred through adaptive speciation in allopatry (Fig. 8), a model that may be widely applicable in the plant kingdom, particularly in groups inhabiting oceanic or habitat islands (Levin 2000; Waser and Campbell 2004). Although the quartz habitat in the contemporary Knersvlakte landscape has clear spatial structure, geological evidence suggests that it may have been even more islandlike in the recent past (De Beer et al. 2002). The quartz-rich Precambrian rocks, from which the quartz fields are derived, were overlain by an extensive sedimentary deposit during the Pliocene and Pleistocene (about one million years ago). Exposure and continuous expansion of the quartz gravel plain habitat has occurred subsequently, through erosion of this surface. Given that the radiation of the Ruschioideae has been dated to the late Pliocene or early Miocene (Klak et al. 2004), it is likely that *Argyroderma* has diversified onto this expanding habitat. Divergence between early populations may have been facilitated by even stronger spatial isolation and subsequent range expansions may have occurred as the extent of the habitat increased through time, resulting in the coexistence of previously isolated taxa differentiated along habitat use and flowering time axes.

Various lines of evidence, in particular the differences described earlier between patterns of genetic structure in the framesii and delaetii groups, point to two main episodes of range expansion and diversification during the history of the genus. First, the specialist group appears to have expanded its range following the initial evolutionary split between populations occupying habitats with dense and sparse quartz cover. Subsequent divergence of these spatially isolated populations has given rise to the genetically and morphologically coherent taxa within the framesii group. Next, there seems to have been widespread range expansion onto neutral pH, higher saline quartz patches following the evolution of the early flowering schedules in the delaetii group. These two colonization episodes result in the familiar pattern of sympatry of the generalist, a later-flowering framesii-group species and an early-flowering delaetii-group species. Similar multiphase models of adaptive radiation have been proposed for cichlids and other well-known vertebrate radiations (Danley and Kocher 2001; Streelman and Danley 2003).

In radiations where spatial isolation has facilitated adaptive speciation, such as on oceanic islands, multiple historical phases involving the evolution of reproductive barriers in allopatry followed by recolonization would promote the accumulation of diversity. However, there is an obvious conflict between colonization ability and the importance of spatial isolation in promoting adaptive speciation. The complex hygrochastic capsules of the Ruschioideae may provide a mechanism for overcoming this conflict. Their complex architecture ensures that the vast majority of dispersal is extremely local, which is essential to facilitate local adaptation in fine-scale habitat mosaics and to prevent swamping of beneficial

adaptations. However, the highly serotinous capsules, which can contain thousands of seeds, are occasionally dislodged and then become well suited to exploit the rare combinations of events that would allow successful long-distance colonization. Traits that promote the unlikely combination of poor dispersal ability and capacity for long-distance colonization (i.e., successful establishment of viable populations) may well facilitate diversification of the lineages that possess them.

Argyroderma is one of many taxa that have diversified on the quartz gravel plain habitats of the Knersvlakte, which supports 129 known endemic species (Hilton-Taylor 1994). Many of these endemics are members of the Aizoaceae with similar dispersal strategies and life histories, and it is thus likely that the evolutionary mechanisms we propose here for *Argyroderma* may well have played a role in generating the remarkable diversity of this unique flora. In addition, our study provides the first direct evidence supporting previous speculations about the processes underlying the phenomenal radiation of the Ruschiodeae in the winter rainfall desert areas of southern Africa (Ihlenfeldt 1994; Klak et al. 2004). We confirm that genetic isolation between populations is substantial over small spatial scales and strongly influenced by the topographic complexity of the landscape. In addition, we provide correlational support for the idea that adaptive responses to the edaphic and geological complexity of the succulent karoo area have played an important role in generating the remarkable species and growth form diversity within the Ruschioideae.

Acknowledgments

We thank B. Louw and B. Wiese for permission to work on their property; B. Louw for his generous hospitality in the field; A. Spriggs and G. Ellis for field assistance; the Leslie Hill Institute for Plant Conservation for providing a base in Cape Town; S. Macdonald for help with the R-package; R. Cowling, P. Desmet, and U. Schmiedel for useful discussions; and D. Campbell, E. Conti, M. Mort, and an anonymous reviewer for insightful comments on the manuscript. The Western Cape Nature Conservation provided permits to conduct the work, and financial support was provided by the National Research Foundation, South Africa, and the University of California Irvine. Portions of this work were submitted in partial fulfillment of a Ph.D. at University of California Irvine.

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Corresponding Editor: E. Conti