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Space-time diversification of *Androcymbium* Willd. (Colchicaceae) in western South Africa

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Abstract. We examined patterns of cpDNA RFLP variability using 21 restriction endonucleases in 21 populations of *Androcymbium* that represent 12 endemic species distributed in the winter rainfall areas of South Africa to explore the diversification of the genus in its area of maximum species diversity. Our results are supportive of a diversification landscape characterized by continued opportunistic short-range invasion, naturalization, and rapid speciation, in which the selective action of the different environments where *Androcymbium* species occur determined their colonization success and subsequent short-range geographic expansion. The historical presence of fire, the constraint imposed by the low concentration of nutrients throughout southwestern South Africa and the different reproductive capabilities of *Androcymbium* species have also likely stimulated species' diversification. Our divergence time estimates bolster the view that speciation of South African *Androcymbium* initiated in the late Eocene, intensified in the Oligocene and proceeded more sporadically during the Miocene. These chronological estimates also substantiate the previous hypothesis that most lineages of *Androcymbium* in South Africa are much more ancient than their North African relatives, whose diversification began in the late Miocene-early Pliocene.

Key words: *Androcymbium*, Colchicaceae, Cape Floristic Region, cpDNA RFLPs, endemics, diversification.

Androcymbium Willd. consists of about 50 species of hermaphroditic, cormose monocots that pioneer open arid or semi-arid habitats throughout Africa. Although some populations of the genus have been documented in central eastern Africa (Tanzania, Kenya and Ethiopia), most species are restricted to arid areas of northern and southern Africa. *Androcymbium*'s distribution is considered to be a geographic disjunction among these two regions of the continent (Burtt 1971), but this general pattern of occurrence has many other relevant examples in a diversity of vascular plant families (Verdcourt 1969, De Winter 1971, Jürgens 1997).

The 28 species of *Androcymbium* that occur in southern Africa (Leistner 2000) deserve particular attention for three reasons. First, these species offer an excellent opportunity to explore the relative influence of different abiotic factors on organismal diversification. Ten species of *Androcymbium* are endemic to the

Cape Floristic Region (CFR) (Goldblatt and Manning 2000), which harbors one of the highest levels of floristic diversity in the world (Bond and Goldblatt 1984, Cowling et al. 1992, Goldblatt and Manning 2000). Although several studies have implicated the edaphic diversity of the Cape Region as the main cause of this remarkable floristic richness (Linder 1985, Cowling et al. 1992), much remains to be understood about the mechanisms that influenced speciation in this area (Rourke 1982, Goldblatt 1978, Linder 1985). Despite the abundance of ecological and geological data for South Africa, there is still a glaring paucity of studies that put this information into a phylogenetic context to understand the causes of the exceptional floristic diversity in this region.

A second topic of interest concerning *Androcymbium* species in South Africa relates to their diversification. This area is the nucleus of species diversity of the genus and, presumably, its ancestral area of distribution (Caujapé-Castells et al. 1999, 2001). Thus, understanding the mechanisms that influenced speciation in this area is likely to clarify the causes of the sharp differences between the South African species and their North African congeners. In contrast to the North African species, which are restricted to very similar edaphic zones and exhibit low morphological and molecular variation (Pedrola-Monfort 1993; Pedrola-Monfort and Caujapé-Castells 1994, 1996; Caujapé-Castells et al. 1999), South African species of *Androcymbium* are distributed along a wide edaphic gradient that embraces a variety of climatic conditions, and they exhibit high morphological (Membrives 2000, Membrives et al. 2001a) and molecular (Caujapé-Castells et al. 1999, 2001; Membrives et al. 2001b) heterogeneity. Differences in temperature and rainfall are particularly evident between the South African biomes where they are usually found: the sclerophyllous heathlands and the succulent Karoo. To prevent further confusion, our use of 'biome' throughout the paper will refer to ''broad ecological units that represent major life zones extending over large natural areas'', as characterized in Rutherford (1997).

Third, these two biomes are generally under-collected and some of the most diverse groups (especially Mesembryanthemaceae and Liliaceae *sensu lato* like *Androcymbium*) are poorly understood and require major revision (Milton et al. 1997). *Androcymbium* species from the winter rainfall areas of western South Africa feature considerable morphological plasticity, which suggests adaptation to ecological heterogeneity and has caused numerous problems and controversies for species recognition. For instance, the southern African circumscription of *Androcymbium* consists of about 45 taxa according to Müller-Doblies and Müller-Doblies (1998), Pedrola-Monfort et al. (1999a, b) and Membrives (2000), but only of 28 according to Leistner (2000).

The objective of this paper is to combine ecological and geological information with phylogenetic analyses of cpDNA restriction site data to investigate the diversification of *Androcymbium* in western South Africa.

Materials and methods

Plant material. The study includes 21 populations that represent 12 species of the genus *Androcymbium* distributed in sclerophyllous heathlands and succulent Karoo biomes of the winter rainfall areas of western South Africa (Fig. 1, Table 1).

The southern African taxa of *Androcymbium* are hermaphroditic, cormose monocots with androecial nectaries that exhibit various degrees of vegetative reproduction and seed dormancy. The most widespread species (*A. roseum*) occurs between the Limpopo river in Messina (northern province of South Africa) and the Kunene river in Angola, embracing portions of Zambia, North Rhodesia and Botswana. All other southern African species are much more restricted in range, possibly due to limitations in their ecological amplitude. In western South Africa, *Androcymbium* occurs predominantly in sclerophyllous heathlands and in succulent Karoo biomes. Although our sampling includes a thorough geographical and taxonomic representation of the known populations of *Androcymbium* in western South Africa, we

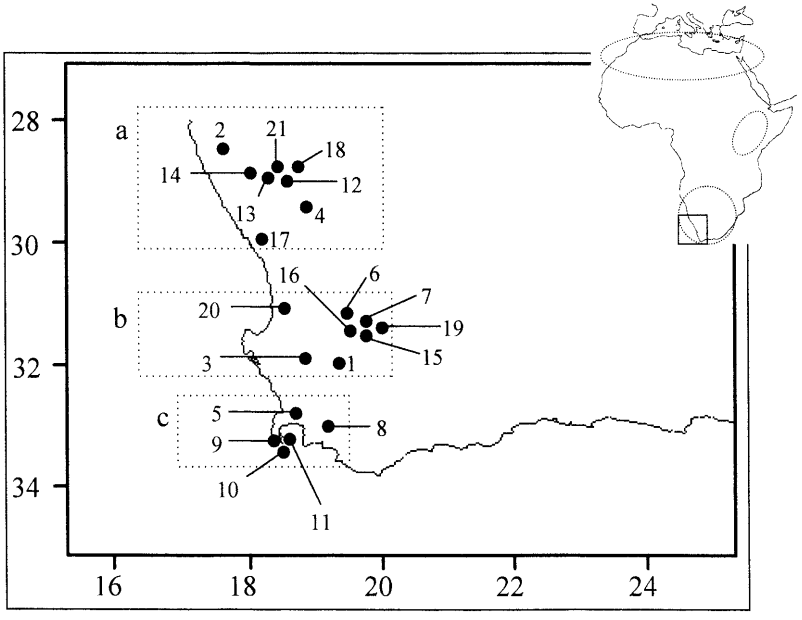

Fig. 1. Geographical location of the populations sampled. Numerical codes correspond to those in Table 1. The three areas (*a*, *b*, *c*) framed in dashed lines are arbitrary subdivisions of this geographical space. Dotted ellipses refer to the general distribution of genus *Androcymbium* in Africa

must bear in mind that these two biomes are generally under-collected.

The sclerophyllous heathlands are associated with sandy, white soils that are poor in nutrients and have a winter rainfall over 250 mm/year. The succulent Karoo is characterized by a low altitude, arid and hot landscape, which can be rocky or sandy (Taylor 1978, Acocks 1988). This biome is unusually rich in chamaephytes and geophytic petaloid monocots like *Androcymbium* (Evenari et al. 1985, Goldblatt 1978), and it has a remarkable scarcity of tall shrubs, trees and grasses (Milton et al. 1997).

We collected about 2 kg of soil from the site of each sampled population. These soil samples were dried and subjected to edaphic analyses for 16 variables (13 quantitative + 3 qualitative) as described in Membrives et al. (2001b). The quantitative values were used to calculate edaphic distances between pairwise combinations of populations with the option 'taxonomic distance' in NTSYS (Rohlf 1993).

DNA methods. DNA isolation, digestion with 21 restriction endonucleases and filter hybridizations were carried out as described in Jansen and Palmer (1987) and Caujapé-Castells et al. (1999). For phylogenetic analyses, we selected three outgroups from the Colchicaceae based on their close phylogenetic relationship to *Androcymbium* (Buxbaum 1936, Nordenstam 1982, Persson 1993): two populations of *Colchicum lusitanum* and a population of *Merendera montana* (Table 1). Our dataset was a subset of that used in Caujapé-Castells et al. (1999) and is available from JCC on request (julicaujape@granca.step.es) and at http://www.biosci.utexas.edu/IB/faculty/jansen.htm.

Parsimony analyses were performed using heuristic searches with 100 random replicates and the TBR branch-swapping option in PAUP* version 3.1.1 (Swofford 1991) with MULPARS and ACCTRAN optimization using Wagner (Farris 1970) parsimony. Bootstrap values (Felsenstein 1985) were obtained from 100 replicates using a heuristic search with random addition sequence of taxa, MULPARS, ACCTRAN optimization and TBR branch swapping.

Differences in rates of cpDNA evolution were evaluated by pairwise comparisons involving 13 representative populations of *Androcymbium* from independent branches of the phylogeny and the outgroup *Merendera montana* (see taxa in bold in Fig. 2) using the two-tailed Wilcoxon matched-pair signed rank test (Templeton 1983). Calculations of sequence divergence between all pairwise combinations of these populations were carried out following Nei and Li (1979). These values were used to estimate divergence times with a slow (0.70%) and a fast (0.1%) average divergence rate per million years (Parks and Wendel 1990, Wendel and Albert 1992). The average between the divergence times resulting from each rate of change was calculated at eleven major nodes in the tree to estimate the

Table 1. Geographical distribution of the populations examined in this study. Numeric codes under N correspond to the locations of these populations in Fig. 1. Codes in parentheses after the stand codes are the voucher specimens deposited in the herbarium of the Marimurtra Botanical Garden. Collector codes are JCC: Juli Caujapé-Castells; JG: Jordi Gibert; JPM: Joan Pedrola-Monfort; MV: Magdalena Vicens. Asterisks signal where edaphic samples were taken

N	Species/Stand code (voucher)	Locality	Collectors
	A. albanense subsp. *clanwilliamense* Schönland		
1	ALB-PK* (JBMM 1386)	Pakhuispass	JCC, JG, JPM
	A. bellum Sclechter & K. Krause		
2	BEL-VI (JBMM 1378)	Vioolsdrift	JCC, JG, JPM
	A. irroratum Schlechter & K. Krause		
3	IRR-VP (JBMM 1387)	Vanrhynsdorp	JCC, JG, JPM
	A. walteri Pedrola, Membrives & J. M. Monts.		
4	WAL-ST* (JBMM 1651)	Steinkopf	JCC, JG, JPM
	A. eghimocymbion U. M, II.-Doblies & D. M, II.-Doblies		
5	EGH-CI* (JBMM 1662)	Citrusdale	JCC, JG, JPM
	A. cuspidatum Baker		
6	CUS-CA1* (JBMM 1391)	Around Calvinia	JCC, JG, JPM
7	CUS-CA2	Around Calvinia	JCC, JG, JPM
8	CUS-MO (JBMM 1367)	Montagu	JCC, JG, JPM
	A. austrocapense U. M, II.-Doblies & D. M, II.-Doblies		
9	AUS-GH (JBMM 1371)	Cape of Good Hope	JCC, JG, JPM
10	AUS-WP1	Whale Point	JCC, JG, JPM
11	AUS-WP2* (JBMM 1370)	Whale Point	JCC, JG, JPM
	A. circinatum Baker		
12	CIR-N1 (JBMM 1389)	Nababeep	JCC, JG, JPM
13	CIR-N2	Nababeep	JCC, JG, JPM
14	CIR-SP* (JBMM 1388)	Springbok	JCC, JG, JPM
	A. hantamense Engl.		
15	HAN-CA1 (JBMM 1390)	Around Calvinia	JCC, JG, JPM
16	HAN-CA2	Around Calvinia	JCC, JG, JPM
	A. burchellii subsp. *pulchrum* Schinz		
17	PUL-NI* (JBMM 1384)	Nieuwoudtville	JCC, JG, JPM
18	PUL-CA* (JBMM 1385)	Around Calvinia	JCC, JG, JPM
	A. burchellii subsp. *burchellii* Baker		
19	BUR-HX (JBMM 1368)	Hexrivier	JCC, JG, JPM
	A. poeltianum U. M, II.-Doblies & D. M, II.-Doblies		
20	POE-CO (JBMM 1377)	Concordia	JCC, JG, JPM
21	POE-NA (JBMM 1376)	Nababeep	JCC, JG, JPM
OUTGROUP SPECIES			
	Colchicum lusitanum Brot.		
	CLU-CF	Cortes (Spain)	JCC, JG
	CLU-LA	Laína (Spain)	JCC, JG
	Merendera montana (Pourret) P. Fourn.		
	MMO-AN	Ansó (Spain)	MV

historical sequence that gave rise to these species. Pairs of taxa that showed significant rate differences according to the Wilcoxon test were not included in these calculations.

Mantel tests (Mantel 1967) were carried out in NTSYS (Rohlf 1993) to explore the relative influence of isolation by distance and ecological heterogeneity on patterns of diversification in

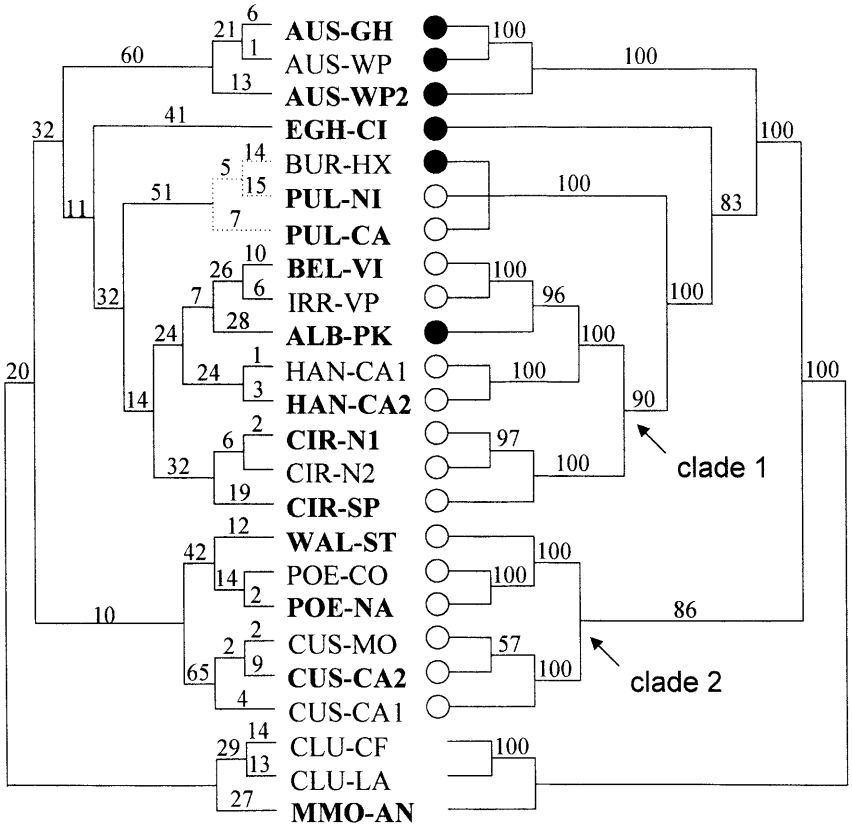

Fig. 2. One of the two most parsimonious Wagner trees (left) and bootstrap tree (right) based on cpDNA restriction data of *Androcymbium*. Dashed lines indicate the branches that collapse in the strict consensus tree. Vegetation zones for each population are indicated by open (succulent Karoo and Karroid types) and closed (sclerophyllous forests) circles. Trees are 776 steps long with a CI = 0.695, HI = 0.305 and RI = 0.867, excluding autapomorphies. Numbers above branches are restriction site changes and amount of bootstrap support for trees (on left and right, respectively). Populations in bold are those used for testing the molecular clock

Androcymbium. This is a non-parametric statistical procedure that calculates the correlation between pairs of distance matrices and tests its significance. Because this test uses all possible pairs of sample locations, it effectively increases the sample size on which inferences are based. Mantel analyses involved three different distance matrices: a genetic distance matrix containing Nei and Li's (1979) nucleotide divergence, a distance matrix between stands based on their respective coordinates in geographical space (Table 1), and an edaphic distance matrix constructed using the option 'taxonomic distance' in NTSYS (Rohlf 1993) after standardizing the values of 13 quantitative edaphic variables. Tests were carried out with 9,999 randomizations.

Results

The g1 statistic for 1,000 randomly generated trees is −0.641, indicating that the data are skewed significantly from random ($P < 0.01$ for g1 = 500 characters and 25 taxa). Therefore, there is considerable phylogenetic signal in our data set (Hillis and Huelsenbeck 1992). Each of the two most parsimonious trees is 776 steps long and has a CI = 0.695, a HI = 0.305 and a RI = 0.867, excluding autapomorphies (Fig. 2). These values indicate low levels of homoplasy in this dataset according to a survey by Sanderson and Donoghue (1996). The cpDNA phylogeny provides strong

support for the monophyly of all species represented by more than one population (*A. austrocapense*, *A. hantamense*, *A. circinatum*, *A. poeltianum* and *A. cuspidatum*).

Whereas some con-specific populations are very similar in terms of restriction site changes (e.g. ANPOE-CO/ANPOE-NA and ANHAN-CA1/ANHAN-CA2 differ only by two and four restriction sites, respectively), many of the species represented by more than one population in our survey exhibit a remarkable number of restriction site changes. In *A. austrocapense*, 35 changes separate AN-AUS-WP from ANAUS-WP2. The two populations of *A. burchellii* subsp. *pulchrum* sampled differ by 27 restriction site changes.

The hypothesis of a uniform molecular clock is rejected in 36 of the 105 comparisons (Table 2). Fourteen of these rejections correspond to combinations between one *Androcymbium* population and the outgroup examined (*Merendera montana*). Of the remaining 22 rejections, 11 correspond to comparisons between populations in different geomorphological areas (Fig. 3) and 11 to the same geomorphological area (the assignment of geomorphological areas to each population follows Boucher and Moll 1981). There are 10 rejections associated with the two clades indicated in Fig. 2. Eight of these correspond to differences between species in these two clades and only two to species within the same clade. The two intra-clade differences fall within clade 1 and correspond to *A. bellum* (AN-BEL-VI)/*A. albanense* (ANALB-PK) and to two populations of *A. circinatum* (ANCIR-N1 and ANCIR-SP). The only molecular rate differences between con-specific populations corresponded to ANCIR-N1 and ANCIR-SP.

Divergence time estimates were calculated at each of eleven nodes in the cpDNA tree (Fig. 3) that represent most of the speciation events for these populations. Four of these values (nodes 1, 6, 10 and 11) correspond to a previous survey that included North African *Androcymbium* populations (Caujapé-Castells et al. 2001). According to these data, the diversification of *Androcymbium* in South Africa started in the late Eocene (node 1, 45.4 ± 11.3 mya) and divided the genus into two major lineages. These stem from nodes 2 and 6 and correspond to the early Oligocene

Table 2. Nei and Li's (1979) nucleotide divergences between 14 representative populations of *Androcymbium* and the outgroup *M. montana*. Species codes correspond to those in Table 1. Asterisks indicate significant differences in the two-tailed Wilcoxon matched-pair signed rank test (Templeton 1983) at $P < 0.05$. Values enclosed in boxes correspond to significant differences within clade 1 or clade 2 in Fig. 2. Underlined values correspond to significant differences between South African populations occurring in different edaphic areas

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. MPY-AN		3.60*	3.70*	2.68*	3.04*	2.54*	3.23*	3.12*	3.97*	4.12*	3.60*	2.39*	3.81*	3.82*
2. ALB-PK			1.15*	3.61*	2.31*	3.77	2.66	2.66	1.53	1.77	1.35	3.37*	1.47	2.63
3. BEL-VI				3.45*	2.36	3.80	3.16	2.80	1.57	1.86	1.72	3.51*	1.76	2.38
4. WAL-ST					2.88	2.39	3.55	3.25*	3.45*	3.59*	3.60*	0.68	3.54*	3.56*
5. EGH-CI						3.12	2.47*	2.11	2.19*	2.26	1.96*	2.84	1.99*	2.40
6. CUS-CA							3.66	3.41	3.63	3.91*	3.82	2.20	3.57	3.77
7. AUS-GH								0.89	2.82	2.84	3.16	3.42	3.30	3.33
8. AUS-WP2									2.36	2.42	2.61	3.00	2.73	3.31
9. CIR-N1										0.53*	1.77	3.26*	1.78	2.04
10. CIR-SP											1.97	3.70*	2.01	2.28
11. HAN-CA2												3.46*	0.66	2.58
12. POE-NA													3.27*	3.71*
13. PUL-CA														2.12
14. PUL-NI														

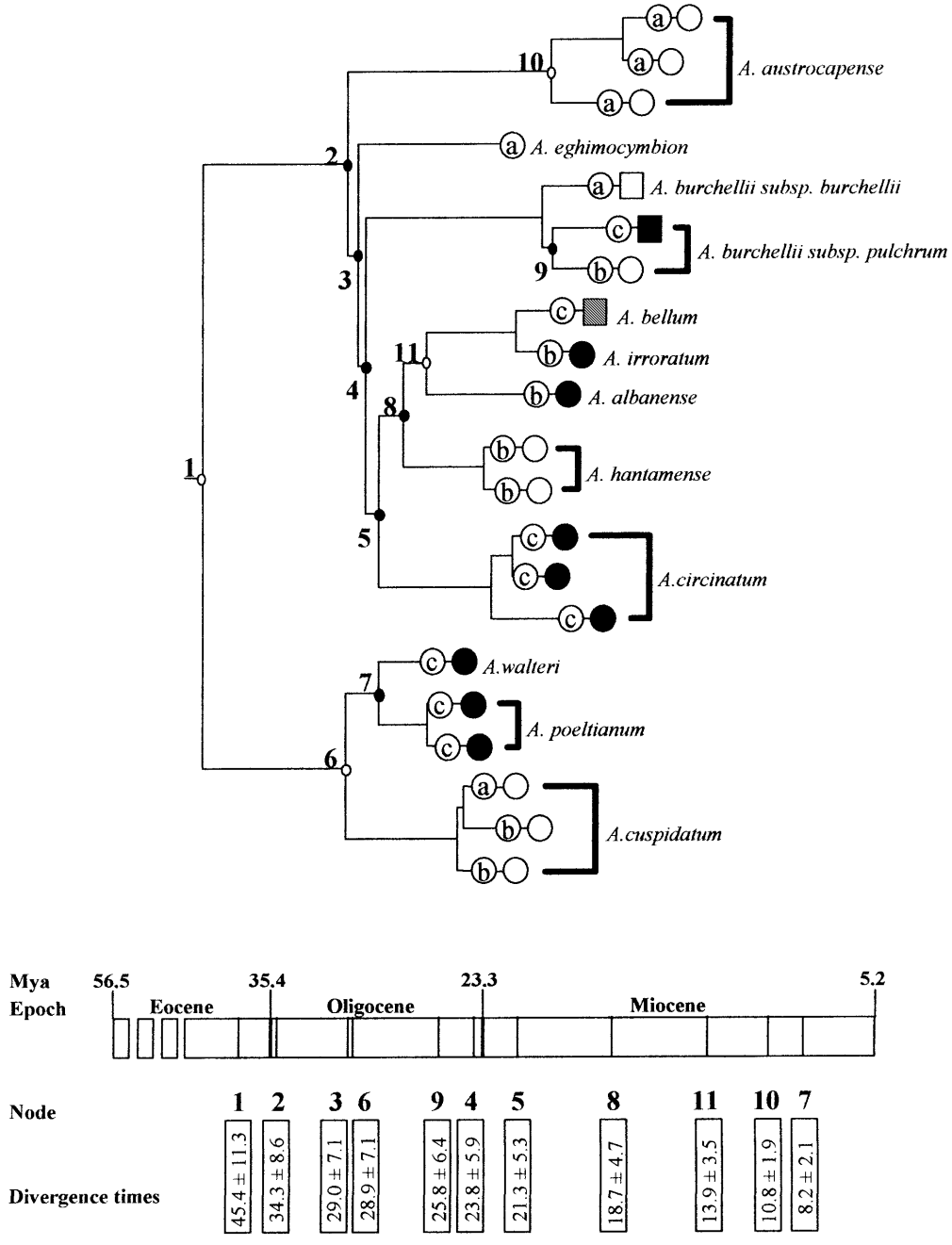

Fig. 3. Phylogenetic relationships among the *Androcymbium* species in ecological space and geological time. Letters in parentheses refer to the geographical areas depicted in Fig. 1. Numbers in boxes associated with the geological chronology (below) are the divergence times (in mya) corresponding to the black and white nodes labelled 1 to 11. The white nodes indicate that time estimates corresponding to a previous work (Caujapé-Castells et al. 2001) that included the North African species of *Androcymbium*. Symbols indicate the geomorphological areas where the populations distribute after Boucher and Moll (1981). Open circles: lithosols and bare rocks; closed circles: colluvial soils with deposition of limestones; open squares: solonetzic and planosolic soils; closed squares: acid sands; stippled squares: calcareous sands and loams. Outgroup taxa have been omitted

(34.3 ± 8.6 mya) and to the middle Oligocene (28.9 ± 7.1 mya), respectively. Our time estimates do not show evidence of any speciation burst in these epochs.

Results of Mantel tests (Table 3) indicate that the correlation between the genetic distance matrix and the edaphic distance matrix is positive (r = 0.164) and that the correlation between the geographical distance and the genetic distance is negative (r = −0.184). None of these values was significant.

Discussion

Differentiation in space. Plant diversification in western South Africa is likely the result of interactions among four factors: sharp climatic variations, steep edaphic gradients, a rich geological history (Linder 1985, Deacon et al. 1992) and the biological and phylogenetic traits of the organisms (e.g. reproductive systems or habitat specialization). As highlighted by Linder et al. (1992), the diversity of possible interactions among these abiotic and biotic factors suggests that the origins and maintenance of species diversity in this region do not fit any general explanation. However, the relative impact of geographical isolation versus ecological selection on plant diversification has been the focus of considerable debate. Rourke (1972) and Goldblatt (1978) report strong evidence for isolation by distance, and they argue that this has been the predominant form of diversification in the Cape Floristic Region (CFR). In contrast, Linder (1985) suggests that speciation in response to marked ecological gradients was prevalent in this area. Recent examples of the latter hypothesis are abundant and span a wide array of plant families (Bruyns and Linder 1991, Kurzweil et al. 1991, Linder and Vlok 1991, Linder 1995, Manning and Linder 1992, Linder and Mann 1998).

Table 3. Mantel correlations (*r*) and significance probabilities (*P*) between pairwise comparisons of edaphic, genetic and geographical distance matrices

	Edaphic	Genetic
Genetic	r = 0.164	–
	P = 0.834	–
Geographical	r = 0.030	r = −0.184
	P = 0.424	P = 0.427

If geographic isolation has been a factor in the diversification of *Androcymbium* in South Africa, we would expect a direct positive relationship between phylogenetic relatedness and geographical adjacency. Although the topology of the cpDNA tree (Fig. 2) illustrates several cases that agree with this pattern, there are a number of relevant exceptions in each of the major clades. *Androcymbium eghimocymbion* (ANEGH-CI, from area 'a' in Fig. 1) and *A. burchellii* subsp. *pulchrum* (ANPUL-NI and ANPUL-CA, from area 'c') are much closer phylogenetically than *A. cuspidatum* and *A. austrocapense* (ANCUS-MO, ANCUS-CA1, ANCUS-CA2, ANAUS-GH, ANAUS-WP and ANAUS-WP2 occur in area 'a') or than *A. burchellii* subsp. *pulchrum* and *A. cuspidatum* (both distributed in area 'c'). Further, Mantel tests cast additional doubt on any general trend toward isolation by distance for *Androcymbium* in this area by showing that the correlation between the geographical distance matrix and Nei and Li's (1979) nucleotide divergence is the only negative value (r = −0.184), yet non-significant. This is noteworthy because *Androcymbium*, like many species occurring in the Cape Floristic Region, is myrmechocorous (Linder 1985, Slingsby and Bond 1985), which likely undermines its dispersal capabilities. Ant dispersal and the burial of seeds facilitate optimal seed establishment through avoiding predation by granivorous rodents (Bond and Breytenbach 1985) at the expense of limiting dispersal range considerably.

Possible explanations for failing to detect this kind of correlation in *Androcymbium* are: (1) post-speciation long-range dispersal, and (2) disruptive selection between geographically close stands. Myrmechocory notwithstanding, there are two other compelling pieces of evidence that allow us to rule out long-range dispersal as a factor in the diversification of the genus. First, the number of known populations

for most species of *Androcymbium* occurring in the surveyed area is very low. For instance, only one population of *A. walteri*, *A. albanense* subsp. *clanwilliamense*, *A. cruciatum* or *A. huntleyi* has been described (Müller-Doblies and Müller-Doblies 1998, Membrives 2000). Second, most conspecific populations are geographically adjacent (e. g., in *A. cuspidatum*, *A. austrocapense*, *A. circinatum*, *A. hantamense* and *A. poeltianum*).

A recurrent conclusion of most biological studies in western South Africa is the implication of environmental heterogeneity in explaining plant species richness. The soil composition of the CFR, with steep edaphic gradients within a narrow area, seems to be especially suited to promote rapid species diversification. According to Axelrod and Raven (1978), the high species diversity in many southwestern South African genera is an adaptive response to the numerous microhabitats that characterize this region. Environmental heterogeneity is reported to have fostered the evolution of numerous habitat specialists and ecologically equivalent species in the Cape Floristic Region (Cowling et al. 1989, 1997). Figure 2 reveals that phylogenetically close species sometimes occur in very different edaphic zones, thereby providing qualitative evidence that environmental differences might be an important factor to explain the diversification of *Androcymbium* in the CFR. Quantitatively, 11 out of the 22 molecular clock rejections correspond to species in different edaphic areas (Table 2), which reveals the relationship between the sharp contrasts of the South African environmental mosaic and the patterns of genetic differentiation. Although we only consider edaphic factors because of their relative stability in space and time, differences among and within clades are evident for other ecological variabes (i.e. temperature, monthly rainfall or humidity). A relationship between biological differentiation and climatic patterns was concluded in an exhaustive cladistic biogeographic analysis of the southern African *Thamnochortus* (Restionaceae) (Linder and Mann 1998), suggesting that speciation was driven by climatic shift. Based on sequence data, Bakker et al. (2000) indicate that aridification of South Africa in the late-Pliocene probably triggered the colonization of different ecological niches by *Pelargonium* (Geraniaceae). The examples of *Androcymbium*, *Thamnochortus* and *Pelargonium* are relevant because they illustrate that aridification may have played an important role in the geographical diversification of the South African Flora. Membrives et al. (2001a) did not detect a significant correlation between edaphic variables and isozyme data in southwestern African *Androcymbium*. Nevertheless, they noted that levels of isozyme variation in these taxa diminish as aridity increases towards the North, especially in self-compatible species. For example, three populations of *A. irroratum* from Eksteenfontein (northwestern Cape, Namaqualand, bordering Namibia) were much less variable than the four populations from the southern Cape (Kwagaskllof, Vanrhynsdorp, Vanrhynspass and Kamiesberg, all of them in the succulent Karoo). The profile of diminishing isozyme variability northwards contrasts with the uniform pattern of cpDNA variation among the species in the northern, central and southern zones surveyed (areas a, b and c in Fig. 1), with averages of 91.25, 90 and 92.6 restriction site changes, respectively. The uniformity in the number of cpDNA changes is also apparent in geographically isolated conspecific populations (e.g., in *A. cuspidatum* and *A. burchellii* subsp. *latifolium*).

The most likely explanation for the discrepancies between allozymes and cpDNA relates to the impact of heterogeneous selective forces on these two molecular markers. Allozyme variation has been reported to be influenced by environmental factors, by genetic drift due to founder effects or to small population sizes and by restricted gene flow (Levin 1978). These three factors converge in the distribution areas of *Androcymbium*. Contrasting ecological milieus may have promoted conspicuous spatial discontinuities among populations of *Androcymbium*, thus increasing their potential for adaptive differentiation by

reducing the levels of gene flow and by fostering the fixation of different isozymic alleles. In contrast, the cpDNA genome is unlikely to have been influenced by selection. Although theoretical studies conclude that if selection acts upon organellar genomes the time of fixation for beneficial genomes must be much shorter than for neutral ones (Tajima 1983, Maruyama and Birky 1991, Milton 1994), there are no empirical reports of physiological consequences of variation in cpDNA.

A second factor that may add to the differences observed between isozymes and cpDNA RFLPs is the different inheritance of these two markers. Significantly, when linked genome inheritance is the case for all markers used in molecular studies, then considerable congruence arises, as found in a phylogeny of *Pelargonium* based on mtDNA and cpDNA sequences (Bakker et al. 2000).

The interpretation of the cpDNA phylogeny that we favor is that the radiation of *Androcymbium*, in western South Africa involves short-range invasion, naturalization and rapid speciation in which the selective action of the different environments determines colonization success. Subsequent to the 'short range expansion' stage, the degree of geographical or ecological isolation and the reproductive capabilities of the different species (Membrives 2000) probably influenced the tempo and mode of differentiation. For instance, isozyme analyses (Membrives et al. 2001b) show that populations of cleistogamous or highly self-compatible *Androcymbium* species (e.g. *A. eghimocymbion*, *A. huntleyi* or *A. irroratum*) may have been more likely to achieve rapid fixation of different genetic variants than self-incompatible or obligate outcrossing species (e.g. *A. bellum*, *A. circinatum*, *A. hantamense* or *A. walteri*).

Differentiation in time. Because the geological history of South Africa is well documented, interpreting the phylogeny of *Androcymbium* in terms of divergence times gives a different perspective on our data and allows us to discuss the probable speciation dynamics of the genus in this area (Fig. 3). Considering that the South African species of *Androcymbium* are closely related and do not exhibit differences in growth habit, it seems safe to assume that rates of evolution among these species are likely to be stable (Li 1993). Therefore, our divergence time estimates can be used confidently to exclude some unlikely diversification scenarios.

The middle Miocene is regarded as the geological epoch with the largest turnover of plant taxa in the Cape Floristic Region (Coetzee et al. 1983). Although the cpDNA phylogeny of *Androcymbium* shows several diversification events in the Miocene, the genus does not seem to have experienced a speciation peak in this epoch. Rather, our divergence time estimates (Fig. 3) support the hypothesis that South African *Androcymbium* diversified in response to a continuous speciation process that started in the late Eocene, intensified in the Oligocene and proceeded more sporadically during the Miocene. This process presumably covered all edaphic areas and was accompanied by differentiation through adaptation. The antiquity of South Africa's edaphic mosaic (Partridge 1997) and the absence of catastrophic change associated with Pliocene-Pleistocene climatic cycles (Scott et al. 1997) suggest that the process was aided by remarkable environmental uniformity through time. The historical presence of fire and the constraint imposed by the low concentration of nutrients throughout southwestern South Africa (Cowling et al. 1989, 1997) have also likely stimulated this high degree of species diversification. The region of Calvinia ('b' in Fig. 1) seems to be particularly well suited to this speciation scenario because of the confluence of morphologically different *Androcymbium* species in a narrow (but geomorphologically diverse) area.

Fossil evidence indicates that the climate in the Cape Region deteriorated rapidly at the end of the Tertiary, changing from tropical to a warm temperate forest climate and eventually to a summer-dry Mediterranean climate (Coetzee 1983, Linder et al. 1992). This process probably extirpated many populations

and species of vascular plants in this region (Linder et al. 1992). Linder et al. (1992) also predict that most groups affected by the sharp climatic shifts in the Cape Region at the Miocene-Pleistocene would be largely well defined, with few, if any, species complexes. A substantial reduction in taxonomic diversity of *Androcymbium* through extinctions at some stage in the geological past is one possible explanation for the high number of steps associated with many branches in the cpDNA phylogeny (Fig. 2). For example, clade 2 is supported by a high bootstrap value of 86%, but its two subclades are separated by 107 restriction site changes. This phenomenon is also observed at the intra-specific level (e.g. the clade grouping the three populations of *A. austrocapense* sampled is supported by a 100% bootstrap value, but ANAUS-GH and ANAUS-WP2 are separated by 40 steps). If extinction has been a factor in the evolutionary history of *Androcymbium* in the Cape Region, then the ubiquity of long branches in our phylogeny could reflect the impact of the end of the Tertiary climatic shifts on the taxonomic makeup of the affected lineages. In this context, the long branches in the cpDNA phylogeny of *Androcymbium* could be the interpreted as (1) an accurate representation of one or more speciation events that did not result in the acquisition of synapomorphies (Bateman 1996); or (2) the effects of extinction on the affected lineages. In either case, the long branches in Fig. 3 may not be resolved by expanding spatial and/or taxonomic sampling. According to the general theoretical framework set forth by Bateman and DiMichele (1994), these "hard" long branches may be associated with saltational speciation events as a consequence of profound morphological transitions and radical phenotypic changes that reflect genome supression events in several lineages. At odds with this prediction, recent molecular evidence (Bakker et al. 2000) shows that the species of *Pelargonium* from the SW Cape underwent a rapid morphological adaptation associated with aridification and the development of a winter rainfall regime without significant change in the DNA sequence. Ongoing molecular research of *Androcymbium* involving sequencing of different regions of the nuclear and cpDNA genomes is expected to provide new data to address this issue (del Hoyo et al., unpubl. data). If genome supression has played a substantial role in the evolution of *Androcymbium*, we would expect to detect numerous deletions and rearrangements in the lineages associated with the long branches in the cpDNA tree.

Alternatively, the high number of steps associated with some lineages in *Androcymbium*'s cpDNA phylogeny may be the result of incomplete taxon sampling. Long branches caused by the absence of data have been termed "soft branches" (Maddison and Maddison 1992, Hoelzer and Melnick 1994), and they represent a series of speciation events that cannot be distinguished because some species have been omitted from the phylogenetic analysis. If sampling is an issue, then breaking up the long branches in the cpDNA phylogeny would require adding more taxa to the analysis. Although we expect to increase the taxonomic sampling of *Androcymbium* in the near future, under-exploration of Liliaceae *sensu lato* in the areas of southern Africa where the genus is distributed (Milton et al. 1997) poses a serious challenge to this major commitment.

The topology of the cpDNA phylogeny (Fig. 2) suggests that not all sclerophyllous habitats where *Androcymbium* species occur represent relictual areas. Although the lineages giving rise to *A. austrocapense*, *A. eghimocymbion* and *A. burchellii* are basal in the phylogeny, *A. albanense* is in a more derived position. Thus, continued evolution in the relictual sclerophyllous vegetation zones must have coincided with colonization and settlement in other sclerophyllous areas. Cowling (1983) suggests that sclerophylls that were able to survive the climatic deterioration at the end of the Tertiary and the Pliocene may have extended their geographic distribution into the areas vacated by the retreating rain forests. We could not estimate the divergence time at the

node including *A. albanense* because the molecular divergence rate between BEL-VI and ALB-PK was significantly different (Table 2). Nevertheless, the topology of the cpDNA tree indicates that this time is within the mid-Miocene, between 18.7 ± 4.7 and 10.8 ± 1.9 mya (nodes 8 and 10 in Fig. 3). Hence, it is unlikely that the *Androcymbium* populations from sclerophyllous areas represent post-Pliocene diversification phenomena. Rather, they are probably more ancient than the climatic deterioration that started in South Africa in the late Miocene.

Conclusions

Our cpDNA phylogeny indicates that the diversification of *Androcymbium* in its area of maximum species diversity is likely the result of a radiation process in which the reproductive capabilities of the different species and the selective action of environmental heterogeneity determined the success of opportunistic short-range colonizations. The genus *Androcymbium* does not seem to have experienced a speciation peak at the middle Miocene, which is regarded as the geological epoch with the largest turnover in plant taxa in the Cape Floristic Region (Coetzee et al. 1983). Rather, our divergence time estimates favor the hypothesis that speciation in *Androcymbium* was not abrupt and proceeded gradually within a wide time period that spans from the late Eocene to the late Miocene. The process was possibly aided by the antiquity of edaphic mosaic and the remarkable climatic stability in South Africa, similar to the situation described in *Phylica* (Richardson et al. 2001a, b).

Several ongoing studies are focussing on analyzing these results further by testing the robustness of the cpDNA phylogeny with other chloroplast and nuclear markers. The first issue of interest concerns the molecular clock rejections between the populations of *A. circinatum* and on the surprisingly high number of rejections where the species *A. walteri* was implicated (eight out of the total 22). Our ongoing investigations will examine if these rate inequities could be related to differences in population sizes or generation times, as is suspected for the North African species based on their remarkably different nuclear DNA contents (J. M. Montserrat and P. Vives, pers. comm.). Second, because uniparental inheritance is predominant in cpDNA (Corriveau and Coleman 1988, Harris and Ingram 1991), the inferred patterns of evolutionary change may differ from those based on nuclear DNA. Although phylogenies based on both genomes are often congruent, disagreements have been reported (Wendel and Doyle 1998), and even extreme situations in which organellar DNA phylogenies lack concordance with biological species' boundaries are known (Powell 1983, Ferris et al. 1983, Avise et al. 1983).

Finally, the hypotheses presented here are for a single genus. Future studies of groups in this region should soon provide the needed tests of the generality of the model proposed for *Androcymbium*.

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