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Author(s): Juli Caujape-Castells, Robert K. Jansen, Joan Pedrola-Monfort, Nuria Membrives
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Chloroplast DNA Restriction Site Phylogeny of the Genus *Androcymbium* (Colchicaceae)

JULI CAUJAPÉ-CASTELLS¹, ROBERT K. JANSEN

Section of Integrative Biology and Institute of Cellular and Molecular Biology, University of Texas,
Austin, Texas 78712

JOAN PEDROLA-MONFORT and NÚRIA MEMBRIVES

Estació Internacional de Biologia Mediterrània-Jardí Botànic Marimurtra, Passeig Karl Faust 10,
17300 Blanes, Girona (Spain)

¹Corresponding author: Present address-Departamento de Biología, Universidad de Las Palmas, Campus
Universitario de Tafira, 35017 Las Palmas, Islas Canarias, Spain (caujape@lix.intercom.es) and Jardín
Canario "Viera y Clavijo", Apartado 14 de Tafira Alta, 35017 Las Palmas de Gran Canaria, Spain

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ABSTRACT. We examined phylogenetic relationships among 51 populations representing 18 of the approximately 40 currently recognized species in the genus *Androcymbium* using 559 parsimony informative cpDNA restriction site changes. Our results indicate that the genus is monophyletic and had a southern African origin. According to our estimates of divergence times, species distributed in North Africa arose in the early Miocene from an ancestor related to *A. eucomoides*. The two Canary Island endemics originated from an ancestor related to the North African *A. wyssianum* in the early Pliocene. Strong support for the monophyly of all the species represented by more than one population casts doubt on the inclusion of the six North African species into a *Androcymbium gramineum* complex and provides strong evidence against the possibility of reticulation in these species. Lack of support for the monophyly of the two currently recognized sections (*Androcymbium* and *Erythrostickus*) suggests that the presence of wing-like appendages on the tepals is not a reliable taxonomic character for sectional circumscription. Molecular clock estimates emphasize the importance of ecological diversity in the differentiation of South African species. In sharp contrast, the much less pronounced pattern of change among north African species seems to have been fostered by their relatively recent origin and by the uniform ecological conditions that prevail in their areas of geographic distribution.

Androcymbium Willd. (Colchicaceae) consists of about 40 species of hermaphroditic, cormose monocots with androecial nectaries that pioneer open arid or semi-arid habitats in Africa. Although some populations of the genus have been documented in zones of central eastern Africa (Tanzania, Kenya, and Ethiopia), most species are restricted to northern and southern Africa. The six species in northern Africa are currently considered part of the *Androcymbium gramineum* complex (Greuter 1967). Four of them occur in the Mediterranean region: *A. gramineum* (widespread in southern Spain and the Atlantic coast of Morocco), *A. palaestinum* (with two known populations in the Middle East), *A. rechingerii* (widespread in the coastal fringe of Lybia and with one population in the Greek islet of Elafonisos), and *A. wyssianum* (widespread in interior Tunisia, Algeria and Morocco, and along the Atlantic coast of Mauritania). The other two north African species occur in the Canary Islands. *Androcymbium hierrense* has been described in three localities in the

western islands of El Hierro, La Palma and La Gomera. *A. psammophilum* has two known populations in the eastern islands of Fuerteventura and Lanzarote. The two Canarian species differ from their mainland relatives in their much larger size and morphological adaptations associated with self-fertilization. These latter features are especially apparent in *A. psammophilum*, which has stamens that curve towards the pistil.

The remaining species are distributed throughout the Atlantic coast, the central zone and the Indian coast of southern Africa. The most widespread species, *A. roseum*, occurs between the Limpopo river in Messina (northern province of South Africa) and the Kunene river in Angola including portions of Zambia, North Rhodesia, and Botswana. The other southern African species are much more restricted in range, possibly due to limitations in their ecological amplitude.

There has been considerable debate on the taxonomic status and phylogenetic relationships in *An-*

drocymbium since Cavanilles (1801) described the species *A. punctatum* (now known as *A. gramineum*). One taxonomic controversy concerns the sectional circumscriptions. Since Müller-Doblies and Müller-Doblies (1990) eliminated Krause's (1920) section *Degreocymbium*, the genus consists of two sections. Sectional assignments are made exclusively on the basis of the presence (section *Erythrostictus*) or absence (section *Androcymbium*) of wing-like appendages at the base of the tepal blade. Only the six north African taxa and the south African *A. bellum*, *A. dregei*, *A. exiguum*, and *A. roseum* are currently included in section *Erythrostictus* (Müller-Doblies and Müller-Doblies 1990). As more South African species have been described, the taxonomic usefulness of tepal morphology has been questioned because of its considerable variability (Müller-Doblies and Müller-Doblies 1990; Pedrola-Monfort 1993).

Two other factors that complicate taxonomic circumscriptions are the lack of distinctive qualitative characters and the remarkable intra-specific variability of biometrical ones. This was recognized long ago (Maire 1934; Bellot 1965) and has stirred taxonomic debate ever since. Roessler (1974) argued that *A. roseum* (the most widespread southern African species) and the North African *A. gramineum* should be considered conspecific. However, there is now evidence that they are well differentiated (Martín et al. 1993; Pedrola-Monfort 1993). An analysis of capsule size based on a biased sampling misled Kunkel (1977) to conclude that the Canarian *A. psammophilum* was a subspecies of the North African *A. gramineum*. This conclusion is now considered unjustified based on a detailed biometric study (Pedrola-Monfort and Caujapé-Castells 1994). Morphological variability has also made taxonomic identification of southern African species difficult (Pedrola-Monfort et al. 1999).

At present, hypotheses for relationships among *Androcymbium* species are based only on morphological evidence and are incomplete. This is due to the paucity of qualitative morphological characters and the lack of data for many species in southern Africa. The most relevant phylogenetic discussions to date concern the relationships among the species in Northern and southern Africa, and the origin of the Canary Island species. Recent studies of palynology (Martín et al. 1993) and karyology (Margeli et al. 1998) provide the only data bearing on the South-North relationship. Bramwell (1976) suggested a vicariance relationship between the Canary Island endemic *A. psammophilum* and the North African *A. gramineum* based on morphological simi-

larity. In contrast, Pedrola-Monfort and Caujapé-Castells (1994, 1996) hypothesized that the two Canarian species *A. hierrense* and *A. psammophilum* have a closer phylogenetic affinity to the mainland *A. wysianum* on the basis of evidence from allozymes and morphology. In both studies, these conclusions were reached in the absence of data for southern African species. Thus, the origin of *Androcymbium* in the Canarian archipelago is still unresolved.

Clearly, incomplete taxon sampling and the very limited number of morphological characters have prevented a clear understanding of interspecific relationships in *Androcymbium*. This paper aims to provide the first phylogenetic hypothesis for the genus based on chloroplast DNA (cpDNA) restriction site changes. This phylogeny will be used to improve our understanding of relationships within and between the two main distribution areas of *Androcymbium* and to examine the origin of the Canary Island species.

MATERIALS AND METHODS

Sampling. We sampled 51 populations representing 19 species of the genus *Androcymbium*. Except for the populations of *A. rechingerii* along the coast of Libya (which could not be collected because of political problems) and one population of *A. psammophilum* in Lanzarote (from which sufficient DNA could not be obtained), this sampling includes all known populations of the six *Androcymbium* species occurring in north Africa and most of the populations representing 12 of their congeners in the Atlantic coast and western mainland of South Africa (Fig. 1, Table 1). Three outgroups from the Colchicaceae were included: two populations of *Colchicum lusitanum* and a population of *Merendera pyrenaica* (Table 1). They were selected on the basis of their close phylogenetic relationship to *Androcymbium*. The geographic distribution of *Merendera* overlaps with that of *Androcymbium*, whereas *Colchicum* is Eurasian.

DNA Isolation, Restriction Enzyme Digestion, and Filter Hybridization. For each population, total genomic DNA was isolated from 3–8 g of fresh material (maintained in the greenhouses of the Botanical Garden Marimurtra in Blanes, Spain) using the standard CTAB technique (Doyle and Doyle 1987). Due to the small plant size of several species, more than one specimen had to be used for some of the populations. Further purification of DNA was

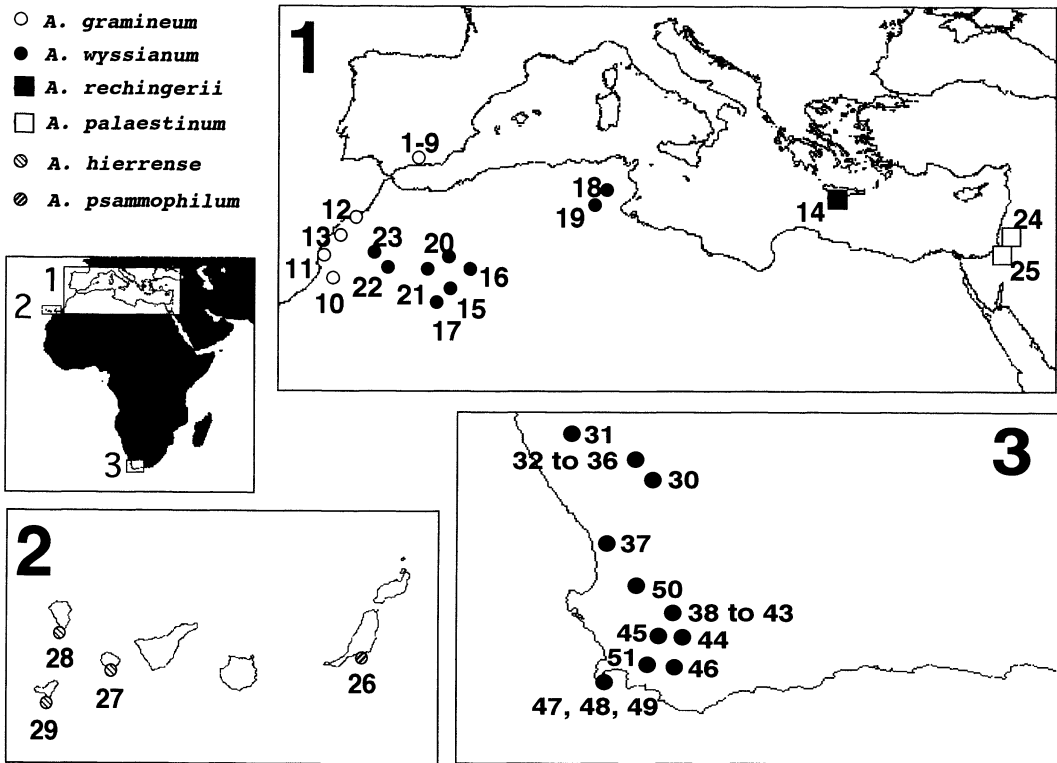


FIG. 1. Geographical position of the populations of *Androcymbium* sampled in 1) north African Mediterranean fringe; 2) Canary Islands; and 3) Atlantic coast and western mainland of South Africa. Populations belonging to different northern African species (numbered 1 to 25) have been given different symbols. Numbers correspond to those in Table 1.

achieved using CsCl/ethidium bromide gradients (Sambrook et al. 1989). Cesium cleaned DNA was digested with 21 restriction endonucleases: 13 six base-pair cutters (*Ava* I, *Ava* II, *Ban* II, *Bgl* II, *Cla* I, *Dra* I, *Eco*O109 I, *Eco*R I, *Hinc* II, *Nci* I, *Nsi* I, *Xba* I and *Xmn* I), one five-cutter (*Bst*N I) and seven four base-pair cutters (*Bst*U I, *Hae* III, *Hha* I, *Hinf* I, *Msp* I, *Rsa* I and *Taq* I). Separation of the resulting fragments in agarose, southern blotting, and subsequent hybridization with radioactively labeled *Oncidium excavatum* cpDNA probes (Chase and Palmer 1989) followed Jansen and Palmer (1987). Chloroplast DNA restriction site patterns were visualized and scored by exposing nylon membranes to X-ray film.

Data Interpretation. Low levels of sequence divergence and the paucity of length variation made it possible to interpret fragment patterns without constructing restriction site maps (Jansen et al. 1998). In cases in which the cause of fragment differences could not be determined (e.g., when a site

gain resulted in fragments too small to be observed), restriction site differences were scored by inferring the presence of small bands. When restriction site differences could not be distinguished from length variants by fragment patterns alone, restriction digests for multiple enzymes from the same region of the chloroplast genome were examined to assure that a single length difference was not scored more than once.

Variable restriction sites were scored as present (1) or absent (0). Length changes were scored as the hypothesized ancestral length (0) or derived length (1) using the outgroups (see next section).

The resulting restriction-site data matrix has been deposited with TreeBASE (<http://www.herbaria.harvard.edu/treebase/>) and is also available from the first author on request.

Phylogenetic Analyses. Parsimony analyses were performed using heuristic searches with 100 random replicates and the TBR branch-swapping option in PAUP* version 4d64 (D. Swofford, with

TABLE 1. Geographical distribution of the populations examined in this study. Numeric codes labelled N correspond to the locations of these populations in Fig. 1. Collector codes are JCC: Juli Caujapé-Castells; JG: Jordi Gibert; JMM: Josep María Montserrat; JPM: Joan Pedrola-Monfort; MA: M. Avishai; MV: Magdalena Vicens; YT: Y. Tankus. New species (n. sp.) are described in Müller-Doblies and Müller-Doblies (1998) and Pedrola-Monfort et al. (1999). Voucher specimens are deposited in the herbarium of the Marimurtra Botanical Garden. See note added in proof (p. 597) regarding new names for several of these species.

N	Population code	Locality	Collectors
<i>A. gramineum</i> (Cav.) McBride			
1	GRA-BC	Almeria	JCC, JPM
2	GRA-CL	Almeria	JCC, JPM
3	GRA-CP	Almeria	JCC, JPM
4	GRA-SC	Almeria	JPM
5	GRA-CH	Almeria	JCC, JPM
6	GRA-EB	Almeria	JPM
7	GRA-ES	Almeria	JPM
8	GRA-LM	Almeria	JPM
9	GRA-PM	Almeria	JCC, JPM
10	GRA-AH	Ain Harrouda	JPM
11	GRA-CB	Cap Bedouza	JPM
12	GRA-CA	Casablanca	JPM
13	GRA-OU	Oualidia	JPM
<i>A. rechingerii</i> Greuter			
14	REC-EL	Elafonisos	JPM
<i>A. wyssianum</i> Beauverd & Turrettini			
15	WYS-TI	Taghit-Igli	JMM, JPM
16	WYS-AO	Ain Ouarka	JPM
17	WYS-IG	Igli	JPM
18	WYS-N1	Nefta 1	JCC, JPM
19	WYS-N2	Nefta 2	JCC, JPM
20	WYS-FB	Fonts Bleus	JCC, JPM
21	WYS-CF	Fonts Bleus' track	JCC, JPM
22	WYS-EF	Erfoud	JCC, JPM
23	WYS-ER	Er Rachidia	JCC, JPM
<i>A. palaestinum</i> Baker			
24	PAL-BS	Beit Shean	YT
25	PAL-DI	Dimona	MA
<i>A. psammophilum</i> Sventenius			
26	PSA-FU	Fuerteventura	JPM
<i>A. hierrense</i> Santos			
27	HIE-GO	Gomera	JPM
28	HIE-LP	La Palma	JPM
29	HIE-HI	Hierro	JPM
<i>A. caujapei</i> Pedrola, Membrives & J. M. Monts. (n. sp.)			
30	CAU-ST	Steinkopf	JCC, JG, JPM
<i>A. bellum</i> Schlechter & K. Krause			
31	BEL-VI	Villesdorp	JCC, JG, JPM
<i>A. poeltianum</i> U. M, II.-Doblies & D. M, II.-Doblies			
32	POE-CO	Concordia	JCC, JG, JPM
33	POE-NA	Nababiep	JCC, JG, JPM
<i>A. guttatum</i> Baker			
34	GUT-N1	Nababiep	JCC, JG, JPM
35	GUT-N2	Nababiep	JCC, JG, JPM
36	GUT-SP	Springbok	JCC, JG, JPM

TABLE 1. Continued.

N	Population code	Locality	Collectors
<i>A. latifolium</i> Schinz			
37	LAT-NI	Nieuwodtville	JCC, JG, JPM
<i>A. cuspidatum</i> Baker			
38	CUS-CA1	Around Calvinia	JCC, JG, JPM
39	CUS-CA2	Around Calvinia	JCC, JG, JPM
<i>A. pulchrum</i> Schltr. & K. Krause			
40	PUL-CA	Around Calvinia	JCC, JG, JPM
<i>A. hantamense</i> Engl.			
41	HAN-CA1	Around Calvinia	JCC, JG, JPM
42	HAN-CA2	Around Calvinia	JCC, JG, JPM
43	HAN-CA3	Around Calvinia	JCC, JG, JPM
<i>A. albanense</i> Schönland			
44	ALB-PK	Pakhuispass	JCC, JG, JPM
<i>A. burchellii</i> Baker			
45	BUR-VP	Vanrhynsdorp	JCC, JG, JPM
<i>A. cuspidatum</i> Baker			
46	CUS-MO	Montagu	JCC, JG, JPM
<i>A. eucomoides</i> Willd			
47	EUC-GH	Cape of Good Hope	JCC, JG, JPM
48	EUC-WP1	Whale Point	JCC, JG, JPM
49	EUC-WP2	Whale Point	JCC, JG, JPM
<i>A. muellerium</i> Pedrola, Membrives & J. M. Monts. (n. sp)			
50	MUE-HX	Hexrivier	JCC, JG, JPM
<i>A. clanwilliamense</i> Pedrola, Membrives & J. M. Monts. (n. sp)			
51	CLA-CI	Citrusdale	JCC, JG, JPM
<i>Colchicum lusitanum</i> Brot.			
	CLU-CF	Cortes de la Frontera (S Spain)	JCC, JG
	CLU-LA	Laina (S Spain)	JCC, JG
<i>Merendera pyrenaica</i> (Pourret)P. Fourn.			
	MPY-AN	Ansó (Pyrenees)	MV

permission) with MULPARS and ACCTRAN optimization. Phylogenetic analyses were performed using both Wagner (Farris 1970) and Dollo (Le-Quésne 1974) parsimony. Although we agree with Albert et al. (1992) that Dollo parsimony is inappropriate for phylogenetic analyses of restriction site data, we used this method as an exploratory tool to examine the effects of the extremes of weighting (Dollo and Wagner) on the resulting tree topologies (Holsinger and Jansen 1993). For the Dollo analysis, the 52 length variants were coded as unordered (Holsinger and Jansen 1993). Bootstrap values (Felsenstein 1985) were obtained from 100 replicates using 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 24 representative populations on the cladogram (see asterisks in Fig. 2) using the two-tailed Wilcoxon matched-pair signed rank test (Templeton 1983).

Constraint analyses were performed to examine the number of steps required to force the monophyly of three groups: species in section *Erythrosticktus* (all north African species plus *A. bellum*), all South African species, and members of both sections *Erythrosticktus* and *Androcymbium*. We

Wagner trees. The only differences concern the relationships among some populations within the clades of *A. wyssianum* and *A. gramineum*. Because of this similarity, we did not analyze the data with any other weighting scheme. The g_1 statistic for 1,000,000 randomly generated trees is -0.311 , indicating that the data are skewed significantly from random ($P < 0.01$ for $g_1 = 500$ characters and 25 taxa). Therefore, there is considerable phylogenetic signal in our data set (Hillis and Huelsenbeck 1992).

The strict consensus tree (Fig. 2) shows very different topologies for the species in the two major areas of distribution. The six North African species form a strongly supported monophyletic group that includes two clades (Fig. 2). One of them consists of the Canarian species *A. psammophilum* and *A. hierrense* plus *A. wyssianum* and *A. rechingerii*, and the other includes western African *A. gramineum* and middle-eastern *A. palaestinum*. Populations of *A. gramineum* from Spain and Morocco do not form distinct monophyletic groups, and only some of the Spanish populations are basal in the clade (Fig. 2).

The South African species do not constitute a monophyletic group and they form three clades supported by high bootstrap values (Fig. 2). One of them consists only of the populations of *A. eucomoides* from southwest South Africa, while the other two contain representatives of the three geographical zones sampled (northwest South Africa, middle-west South Africa, and southwest South Africa). The South African *A. eucomoides*, distributed in the Mediterranean climatic region from Cape Town to Port Elizabeth, is sister to all the North African species. The number of evolutionary steps in the South African lineages is substantially higher than in the north African ones (Fig. 3). Constraining the monophyly of section *Erythrostictus* requires 112 extra steps, and making members of both sections *Erythrostictus* and *Androcymbium* monophyletic requires 128 additional steps. Forcing all South African species into a single monophyletic group adds 46 steps to the tree.

Results of the two-tailed Wilcoxon matched-pair signed rank test (Templeton 1983) indicate that the molecular clock can be rejected in 58 of the 276 comparisons (Table 2). None of the rejections involves two North African taxa, 23 of them are comparisons of *Androcymbium* populations with *Merrindera pyrenaica*, and the remaining 35 are combinations of either a North African and a South African species or of two South African species. Six of these involve South African taxa in the same

monophyletic group (see Table 2). Divergence time estimates for the origin of the North African species, the split of North African species into two groups, and the introduction of *Androcymbium* into the Canary Islands give values of 18.5 ± 3.2 million years ago (mya), 8.21 ± 1.4 mya and 4.38 ± 0.8 mya, respectively.

DISCUSSION

Biogeographical Implications. SOUTH AND NORTH AFRICAN DISJUNCTION. One of the tenets of cladistic biogeography is that areas that are positionally plesiomorphic in a cladogram are more likely parts of the ancestral area than positionally apomorphic areas (Platnick 1981). Our cpDNA phylogeny shows unambiguously that *Androcymbium* has a south African origin because the three basal clades are restricted to that region (Figs. 2, 3). Independent ecological and biological evidence gives additional support. Southern Africa is the center of diversity of the genus with about 35 described species versus only six in northern Africa. Morphological studies show that southern African *Androcymbium* species have a much higher diversity (Roessler 1974; Müller-Doblies and Müller-Doblies 1984, 1990; Pedrola-Monfort et al. 1999). Palynological work revealed that diporate and triporate pollen grains coexist in the Abyssinian species *A. striatum* (Schulze 1975) and in southern Africa (Martín et al. 1993), while all north African species are diporate (Martín et al. 1993). Thanikaimoni (1986) and Takh-tajan (1991) argued that in monocots there is an evolutionary trend from monoporate to diporate pollen grains through a triporate intermediate. According to our calculations, the North African species originated approximately 18.5 ± 3.2 mya, in the early Miocene.

The North African species are divided into two groups, *A. gramineum*/*A. palaestinum*, and *A. hierrense*/*A. psammophilum*/*A. rechingerii*/*A. wyssianum* (Figs. 2, 3) that split about 8.21 ± 1.4 mya (late Miocene). The first group is interesting because *A. palaestinum* and *A. gramineum* are not geographically adjacent (Table 1, Fig. 1) and because a close relationship between these two species and *A. rechingerii* has been suggested based on their sharing of exclusive low-frequency alleles (Pedrola-Monfort and Caujapé-Castells 1996). Placement of *A. rechingerii* in the same clade as *A. gramineum* and *A. palaestinum* in the cpDNA tree would require only two extra steps. Therefore, this topology is not strongly incompatible with the allozymic data. The

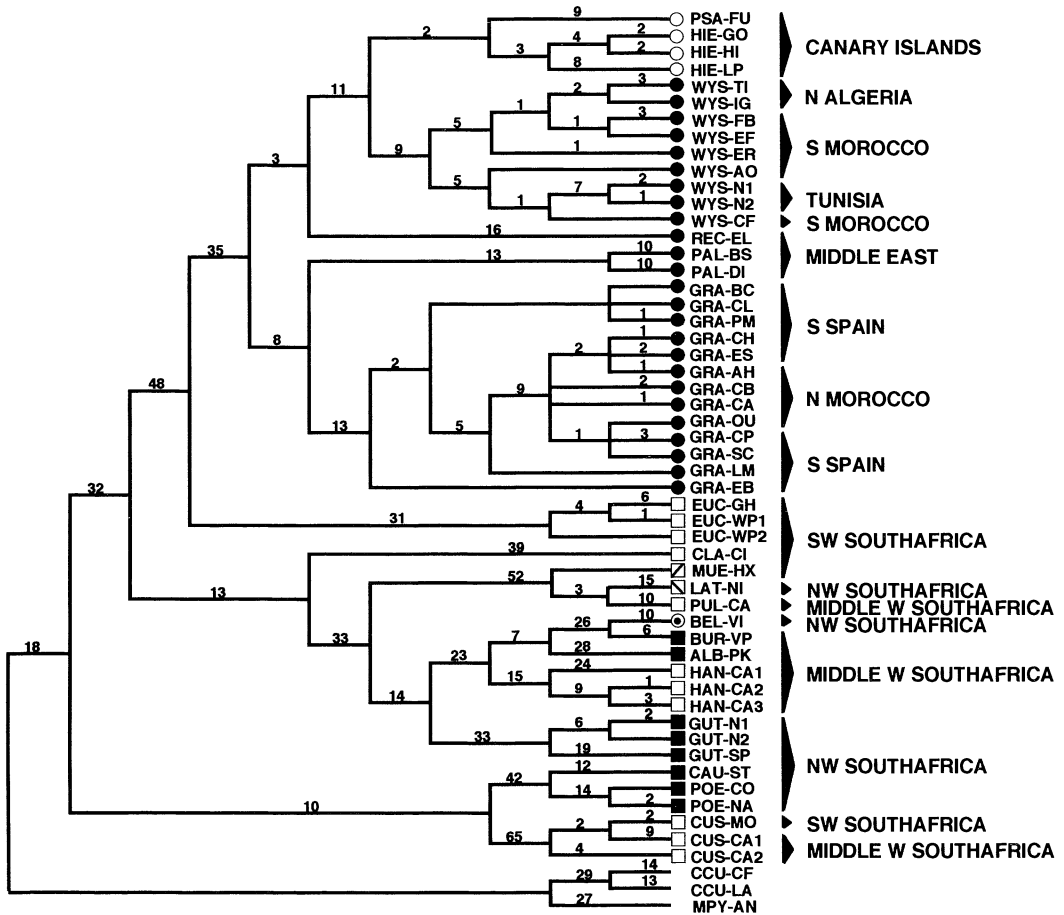


FIG. 3. One of 60 most parsimonious cladograms. Numbers at nodes and along branches indicate number of evolutionary steps as indicated by cpDNA restriction site changes. Symbols at the tip of the branches are general edaphic traits where the populations occur after our soil analyses (Pedrola-Monfort 1993) for north Africa, and after Boucher and Moll (1981) for south Africa. Open circles: basaltic sands; closed circles: calcareous sands; open circles with a dot inside: very shallow calcareous sands and loams; open squares: lithosols and bare rocks; closed squares: weakly developed illuvial soils with lime accumulations in bottom lands; open squares with a downward diagonal line from left to right: mainly acid arenosols; open squares with an upward diagonal line from left to right: solonchic and planosolic soils. Population codes are as in Table 1. See note added in proof (p. 597) regarding new names for several of these species.

context in which the relationship among these three species might have arisen has been discussed extensively through the application of the blocks test (Wilks 1962; Pielou 1974) to the direction-informative subset of cpDNA restriction site changes (Caujapé-Castells et al. in mss.). The picture that emerged from this spatial analysis supports the existence of south-northeast and south-northwest dispersal routes to explain the relationships among *A. palaestinum*/*A. rechingerii*/*A. gramineum*, and *A. wyssianum*/*A. hierrense*/*A. psammophilum*, respec-

tively. The topology of the tree in Figs. 2, 3 is compatible with this dispersal scenario. The Moroccan and Spanish populations of *A. gramineum* are intermixed, with only some of the Spanish populations basal. Thus, *A. gramineum* must have originated in southern Spain and subsequently dispersed, giving rise to the other Spanish and Moroccan populations. Given the uniformity of rates of cpDNA evolution among North African species (Table 2), this is only possible if *A. gramineum* originated from the east. Evidence for a dispersal connection between

TABLE 2. Results of the Wilcoxon matched-pair signed rank test (Templeton 1983) to evaluate the hypothesis of the molecular clock in *Androcymbium*. Codes of species correspond to those in Table 1. Numbers below the diagonal are the number of non-zero sums (bottom) and the minimum difference (top). Symbols above the diagonal stand for non-significant differences (ND) and significant differences at $P < 0.05$ (*) and $P < 0.01$ (**). Values enclosed in boxes correspond to significant differences within the same monophyletic group. Underlined asterisks correspond to significant differences between South African populations occurring in different edaphic areas.

	PSA-FU	HIE-LP	HIE-GO	HIE-HI	WYS-N2	WYS-FB	REC-EL	GRA-CH	PAL-TA	EUC-WP2	EUC-GH	GUT-SP
PSA-FU												
HIE-LP	7											*
HIE-GO	6	6										ND
HIE-HI	5	5	11.5									ND
WYS-N2	12.5	2	2	4								ND
WYS-FB	7	4	4	5								ND
REC-EL	5	5	5	7	6							ND
GRA-CH	6	7	7	7	5							ND
PAL-TA	6	7	7	7	5							ND
EUC-WP2	16	10.5	14	14	16.5	11						ND
EUC-GH	8	6	7	7	9	8						ND
GUT-SP	12	4	4	4	21	27.5	7					ND
GUT-N1	8	5	5	5	9	10	7					ND
BEL-VI	22	16	16	25	10	20	16.5	22				ND
	9	8	10	10	6	9	8	10				ND
	7	31	26	26	21	19.5	35	26	25			ND
	11	11	10	10	10	10	12	12	10			ND
	7	12	12	12	10.5	11	27	12	16	5		ND
	9	9	9	9	10	10	12	10	11	6		ND
	5.5	22.5	18	18	27	33	14.5	24	24.5	33.5	22	
	11	11	10	10	11	11	9	10	11	13	12	
	21	37	44	44	33.5	33.5	36.5	25.5	36	32.5	29.5	0
	13	12	13	13	12	12	12	11	12	11	11	9
	10.5	28	22	24.5	20.5	30	34.5	32.5	35.5	20	23.5	23.5
	10	11	11	10	9	11	12	11	12	9	11	10

TABLE 2. Continued.

	GUT-NI	BEL-VI	ALB-PK	HAN-CA3	PUL-CA	PUL-NI	CLA-CI	CUS-CA1	HAN-CA1	POE-NA	CAU-ST	MPY-AN
GRA-CH	ND	ND	ND	ND	ND	ND	ND	ND	ND	*	*	**
PAL-TA	ND	ND	ND	ND	ND	ND	ND	ND	ND	*	*	**
EUC-WP2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	**
EUC-GH	ND	ND	ND	ND	ND	ND	*	ND	ND	ND	ND	**
GUT-SP	**	ND	ND	ND	ND	ND	ND	ND	ND	**	**	**
GUT-NI		ND	ND	ND	ND	ND	ND	ND	ND	*	*	**
BEL-VI			**	ND	ND	ND	ND	ND	ND	**	**	**
ALB-PK	7	8		ND	ND	ND	ND	ND	ND	**	**	**
HAN-CA2	17	18	16.5		ND	ND	ND	ND	ND	**	**	**
	9	9	9				*					
PUL-CA	7	31	17	17		ND		ND	ND	**	**	**
	7	11	8	8			*					
PUL-NI	21	23	22	20.5	23		ND	ND	ND	**	**	**
	12	10	10	10	11							
CLA-CI	24	10.5	12	20.5	14	4	ND	ND	ND	ND	ND	**
	12	11	12	13	12	10						
CUS-CA1	27.5	15.5	18	20.5	23	5	38	ND	ND	ND	ND	**
	12	10	11	12	12	8	12					
HAN-CA1	21.5	18	12.5	15	20.5	5	28	8		ND	ND	**
	10	10	9	10	11	8	11	6		ND	ND	**
POE-NA	9	2	7	4	3	0	10	20	13		ND	**
	11	10	13	11	12	9	9	11	11			**
CAU-ST	8	0	2.5	5	2.5	1.5	19	24.5	8	12		**
	11	10	11	11	11	11	12	12	9	7		**
MPY-AN	0	0	0	0	0	0	0	0	0	0	3	
	13	13	13	12	13	12	12	11	13	10	12	

A. gramineum and the eastern *A. palaestinum* and *A. rechingerii* was identified by Caujapé-Castells et al. (in mss.). The easternmost populations of *A. wyssianum* (the Tunisian WYS-N1 and WYS-N2) are not basal in the clade of this species, which suggests an origin of *A. wyssianum* from the southwest.

ORIGIN OF THE CANARY ISLAND TAXA. The cpDNA tree provides strong support for a sister group relationship between the Canarian endemics *A. hierrense* and *A. psammophilum* and the North African *A. wyssianum* (Fig. 2). Two previous hypotheses have been suggested regarding the origin of the Canary Island *Androcymbium* species. Bramwell (1976) and Kunkel (1977) used morphology to suggest a relationship between *A. gramineum* and *A. psammophilum*. According to these authors, the latter species was the ancestor of the populations of *A. hierrense* in the western islands. Based on allozymes and biometrical measurements, Pedrola-Monfort and Caujapé-Castells (1994, 1996) indicated that the Canarian taxa are phylogenetically much closer to the mainland *A. wyssianum* than to *A. gramineum*, and that the origin of *A. hierrense* and *A. psammophilum* might be due to independent colonization events from *A. wyssianum*.

Although there are a few molecular phylogenies that suggest that some Canarian species are ancestral to related continental taxa (Knox and Palmer 1995; Ray 1995), the general rule is a derived condition of the Canarian taxa. The most remarkable examples to date are provided by the *Sonchus* alliance (Kim et al. 1996a, b), *Echium* (Böhle et al. 1996), genera of the Sempervivoideae (Van Ham et al. 1994, Mes and Hart 1996), and *Argyranthemum* (Francisco-Ortega et al. 1997). The cpDNA tree for *Androcymbium* (Fig. 2) supports the monophyly and the derived position of the two Canary Island species. A single colonization event from an ancestor closely related to *A. wyssianum* would explain the origin of the two Canarian species. Following our divergence time estimates, the first colonization corresponds to 4.38 ± 0.8 mya (early Pliocene). This introduction gave rise to *A. psammophilum* in the eastern islands, and subsequent inter-island colonization and differentiation generated the populations of *A. hierrense* in the western islands of El Hierro, La Gomera, and La Palma. This evidence refutes Bramwell's (1976) and Kunkel's (1977) hypothesis with regard to the relationships of Canarian species. However, it does agree with their assertion that a single introduction into the eastern islands accounts for the origin of both Canarian species. The fact that the eastern Canary Islands are

very close to the continent (100 km from Fuerteventura) adds support to this hypothesis. It is noteworthy that these were the last islands to attain biological stability (Carracedo 1994, 1996). The possible fluctuations in populational sizes of *A. psammophilum* due to the recent volcanic activity in the Eastern Canary Islands (Schmincke 1982) and the action of genetic drift associated with the origin of this species via a founder effect may have influenced the patterns of allozymic variability that led Pedrola-Monfort and Caujapé-Castells (1996) to suggest two introductions of the Canarian species from *A. wyssianum*.

Taxonomic Implications. The cpDNA phylogeny (Figs. 2, 3) also provides important insights into taxonomic relationships in *Androcymbium*. The monophyly of the genus is strongly supported (100% bootstrap value). There is also strong support for the monophyly of all species represented by more than one population (*A. hierrense*, *A. wyssianum*, *A. gramineum*, *A. palaestinum*, *A. eucomoides*, *A. pulchrum*, *A. hantamense*, *A. guttatum*, *A. poeltianum*, and *A. cuspidatum*). This rules out the possibility of reticulation in these species and bears special significance for the six North African taxa, which had been merged into an *Androcymbium gramineum* complex due to their morphological similarity (Greuter 1967). The cpDNA tree confirms the specific status of all six north African taxa.

Three South African clades are strongly supported and constraining the monophyly of a group consisting of all South African species requires 46 extra steps. One of the three clades only includes populations of *A. eucomoides*. This species is sister to the North African species. Such a well-supported relationship (100% bootstrap value) is remarkable because *A. eucomoides* has never been suggested as a close relative of species in section *Erythrostictus*. Indeed, the only sampled South African species belonging to this section (*A. bellum*) is phylogenetically distant from the North African species. Constraining section *Erythrostictus* to be monophyletic requires 112 extra steps. Hence, if this cpDNA tree topology reflects accurately the phylogenetic relationships in *Androcymbium*, the currently accepted sections are not valid. This agrees with Müller-Doblies and Müller-Doblies' (1990) and Pedrola-Monfort's (1993) opinions based on the extensive variability of the single character (tepal wing) that circumscribes the sections.

Alternatively, the cpDNA phylogeny may be giving an inaccurate estimate of the relationships between South and North African species. Negligible

intraspecific cpDNA variation may not be the rule (Powell 1983; Sytsma and Schaal 1985; Kemble 1987; Harris and Ingram 1991; Soltis et al. 1992). If this is a factor in *Androcymbium*, the topology of our cpDNA phylogeny may not be reflecting the sequence of cladogenic events. Recent macromorphological (Roessler 1974), palynological (Martín et al. 1993), and allozymic (N. Membrives unpubl.) studies support a relationship among north African species and *A. bellum* and *A. roseum* (all members of section *Erythrostickus*). Unfortunately, *A. roseum*, which is the most widespread southern African species, was unavailable because of the unstable political situation in its areas of distribution. The possibility of an inaccurate cpDNA restriction site phylogeny could be explained by invoking lineage sorting (Avice et al. 1983; Neigel and Avice 1986; Doyle 1996) coupled with selection influenced by the environment.

Ecological Diversification. Is there evidence of environmental selection in *Androcymbium*'s phylogeny? The hypothesis that environmental heterogeneity promotes species richness at a regional scale is well documented (Richerson and Lum 1980; Shmida and Wilson 1985; Brown 1988; Diamond 1988; Williamson 1988; Cornell 1993; Cowling et al. 1997). Southern Africa has a much higher level of ecological diversity than northern Africa. *Androcymbium* grows in about 70 different ecological zones in South Africa (Acocks 1988) and only two in North Africa (Greenway 1970; Axelrod and Raven 1978). Given this context, it is not surprising that the extent of evolutionary differentiation among South African species in the cpDNA phylogeny is substantially higher than among north African species. A similar pattern has been detected in morphological (Pedrola-Monfort et al. 1999) and allozymic variation (N. Membrives et al. in prep.).

The higher number of steps in the cpDNA tree (Fig. 3) for South African *Androcymbium* species reveals that either they have had much more time to diverge than their North African congeners or rates of cpDNA evolution have been faster. If the former is true, time since origin is likely to be one factor involved in the conspicuous evolutionary differentiation of *Androcymbium* in South Africa. Two lines of evidence support this time hypothesis: long-term evolutionary conservatism of ecologically important traits (Ricklefs and Latham 1992; Cowling et al. 1997), and the increase in angiosperm diversity through the Cretaceous and Tertiary (Knoll 1986).

Nevertheless, given the antiquity and edaphic di-

versity of South Africa (Partridge 1997), the sharp climatic gradients over much of its area (Schulze 1997) and the absence of catastrophic change associated with Plio-Pleistocene climatic cycles (Scott et al. 1997) it is also likely that the pattern and tempo of diversification in *Androcymbium* have been influenced by the environment. Except for the clade including the populations of *A. eucomoides*, the other two clades with south African representatives do not seem to reflect geographical affinities because they contain species from the three sampled zones (Fig. 3). There is also considerable ecological differentiation among three South African clades (Fig. 3). Although we only consider edaphic factors in Fig. 3 because of their relative stability in space and time, differences among the three clades are evident in terms of every other ecological variable (i.e., humidity, monthly rainfall or temperature). Within clades, it is noteworthy that rates of cpDNA change among conspecific populations (*A. guttatum*) and populations in the same edaphic substrates (*A. hantamense* and *A. clanwilliamense*, *A. pulchrum* and *A. clanwilliamense*) differ significantly (Table 2). In addition, conspicuous morphological differences among populations of *A. burchellii* (Membrives et al. in prep.) have been reported, a phenomenon which has also been documented in *Protea* (Rourke 1982; Bond et al. 1992) and *Haemanthus* (Snijman 1984).

Studies of diversity in South Africa show that plant species richness at the regional scale is determined largely by environmental heterogeneity (Cowling et al. 1997). The fact that all cases in which a molecular clock could be rejected in *Androcymbium* (except those involving the outgroup *Merendera pyrenaica*) include South African species (Table 2) may reflect the action of this ecological heterogeneity.

Selection due to environmental differences is an important factor to explain these rate inequities: a molecular clock is rejected in 12 of 21 comparisons involving two South African species from different edaphic areas (Table 2). 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; Mitton 1994). Given that selection seems to have acted on South African *Androcymbium*'s cpDNA, selectively maintained polymorphisms may have persisted much longer, thereby providing much more opportunity for evolutionary differentiation. This is especially likely in southwest Africa, where the edaphic diversity of the landscape has provoked an explosive

diversification within certain lineages that resulted in the evolution of numerous habitat specialists and ecologically equivalent species (Cowling et al. 1989, 1997). The historical presence of fire and the relatively low fertility of the soils in South Africa (Cowling et al. 1997) may also contribute to this high diversification. In sharp contrast, the much more discrete pattern of change among North African species seems to have been fostered by their relative recency and by the uniform ecological conditions that prevail in their areas of distribution.

In the cases where molecular clock rejections involve different South African species, further investigations are required to examine the effect of generation time. The differential aging of the corms is suspected in triggering processes leading to dramatic contrasts in terms of nuclear DNA content among north African species (J. M. Montserrat and P. Vives pers. comm.). In the absence of information of this kind for the South African species, we cannot determine whether an analogous process might be operating.

Conclusions. The cpDNA restriction site phylogeny of *Androcymbium* has provided insights into three crucial issues. First, it indicates that *Androcymbium* has a southern African origin because the three basal clades contain only species distributed in this area. Since these groups must have originated somewhere (i.e., there must have been an area corresponding to the distribution of their ancestor), this poses the question of the origin of southern African taxa. Second, it emphasizes the importance of ecological diversity in the differentiation of southern African species. Finally, it strongly suggests that the Canarian *A. hierrense* and *A. psammophilum* are monophyletic and originated via a single introduction from an ancestor related to *A. wyssianum* that gave rise to *A. psammophilum* in the eastern islands. This finding clarifies the long debated question of the relationships of the Canarian species with mainland Africa.

A thorough appreciation of the inferred relationships and their taxonomic and evolutionary implications can only be gained after comparison with phylogenies based on independent sources of data. For instance, monophyly of a set of chloroplast genomes does not necessarily imply monophyly of the group in which these genomes occur. Ongoing studies using variable nuclear DNA markers (Caujapé-Castells et al. in prep.) and evaluation of morphological and allozymic evidence (Pedrola-Monfort et al. in prep.; Membrives et al. in prep.) are aimed at clarifying these important issues. Until

these studies are completed, the interrelated questions of the biological validity of the currently recognized sections and the relationships between northern African and southern African species will remain unresolved.

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Note added in proof.

Corrections for several species names used here, based on Müller-Doblies and Müller-Doblies (1998). Authorities' names only appear when they are also affected by the change.

Name used here

A. cauajapei
A. quttatum
A. pulchrum Schltr. & Krause
A. burchellii Baker
A. eucomoides Wild.

A. muellerium U. Müller-Doblies & D. Müller-Doblies
A. clanwilliamense Pedrola, Membrives & Monts. (n. sp)
Merendera pyrenaica

Name in Müller-Doblies and Müller-Doblies (1998)

A. walteri
A. circinatum
A. latifolium Schinz
A. irroratum Schltr. & Krause
A. austrocapense U. Müller-Doblies & D. Müller-Doblies
A. burchellii Baker
A. eghimocymbion U. Müller-Doblies & D. Müller-Doblies
Merendera montana