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Relative influence of biological versus historical factors on isozyme variation of the genus *Androcymbium* (Colchicaceae) in Africa

N. Membrives[1], **J. Pedrola-Monfort**[1], and **J. Caujapé-Castells**[2]

[1]Estació Internacional de Biologia Mediterrània – Jardí Botànic Marimurtra, Blanes, Girona, Spain
[2]Jardín Botánico Canario "Viera y Clavijo", Las Palmas de Gran Canaria, Spain

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Abstract. We carried out isozyme electrophoresis in the South African taxa of *Androcymbium* to compare their levels of genetic variation with those reported for their North African congeners and to evaluate the influence of reproductive traits, phylogenetic relationships and environmental histories on the evolutionary dynamics of this disjunction between arid zones of North and South Africa. One possible factor to explain the higher number of alleles detected in South Africa is that diversification in this area started within the early Oligocene, whereas the ancestor of the North African species is more recent and corresponds to the late Miocene. Strong environmental selection and habitat specialization in South Africa probably fostered genetic differentiation between species further in this area. In South African taxa, isozymic variation levels were significantly correlated to breeding systems but not to lineage circumscription (i.e. phylogenetic closeness). The assessment of the relative influence of diverse biotic and abiotic factors on isozyme variation shows that, on a short geological time scale, breeding system is the most reliable predictor of levels of genetic variation. On a longer time frame, represented by the splitting that gave rise to the south–north disjunction of the genus in the late Miocene, the influence of environmental histories of North and South Africa on genetic variation overrides that of breeding systems.

Key words: *Androcymbium*, isozymes, South Africa, environmental histories, reproductive traits, phylogeny.

Species of the genus *Androcymbium* Willd. are cormose geophytic monocots distributed in arid zones of North and South Africa. There are six species of *Androcymbium* in North Africa (1 in Fig. 1A) and approximately 35 taxa have been described from South Africa. Four of the North African species are circum-mediterranean and embrace climatically and edaphically similar mainland areas in South Spain, Morocco, Mauritania, Tunisia, Algeria, Libya and the Middle East. The other two species are Canary Island endemics and occur in the eastern (Fuerteventura and Lanzarote) and western Islands (La Palma, La Gomera and El Hierro), respectively. South Africa is the center of species diversity, with most populations of *Androcymbium* occurring throughout western arid zones of South Africa (Northern Cape and Western Cape) (2 in Fig. 1A). Seven species (3 in Fig. 1A) are distributed in eastern South Africa (Eastern Cape, Lesotho, Kwuazulu, Natal, Swaziland, Gauten, Mpumalanga and Northern Province) and two more species (4 in Fig. 1A) occur in

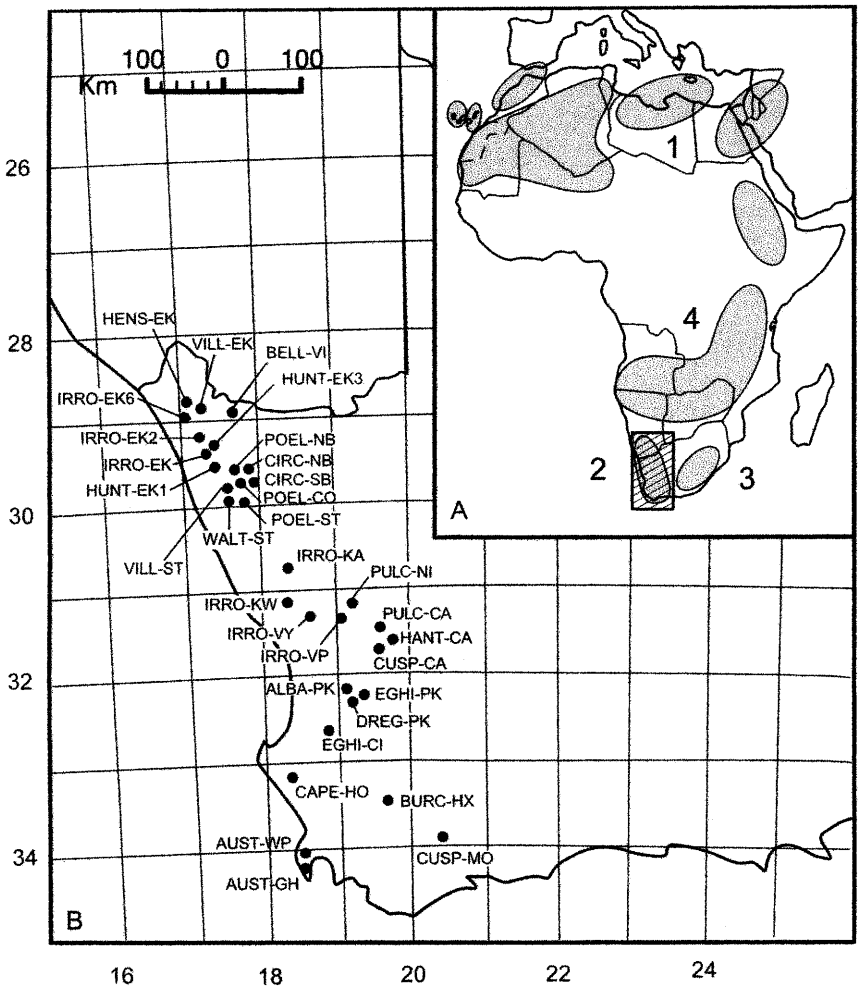

Fig. 1. A Geographical distribution of the genus *Androcymbium*. Numerical codes correspond to the distribution areas and are described in the text. **B** Location of the 32 South African populations of *Androcymbium* sampled for the electrophoretic analysis. Population codes correspond to Table 1

central South Africa (North West Province and Free State) and in Namibia, Angola, Botswana, Zimbabwe, Zambia, Kenya and Ethiopia). Unlike North Africa, South Africa embraces a great diversity of climatic and edaphic microhabitats (Acocks 1988) with an aridity gradient increasing from south to north.

Numerous studies have investigated the relationships among population isozyme variation and biological, ecological and geographical variables using available databases for different organisms (Hamrick et al. 1979, Loveless and Hamrick 1984, Hamrick and Godt 1989). These studies have shown that life form, breeding system and geographic range are, in decreasing order of magnitude, the characteristics that most influence genetic variability in plant populations. However, a substantial amount of that variability could not be explained by these traits alone (Hamrick and Godt 1996) or by combinations among them (Hamrick and Godt 1997). One important shortcoming of these studies is that most of the databases assessed do not consider phylogenetic (Gitzendanner and Soltis 2000) or environmental components. Consequently, little is known about the relationship between phylogenetic relatedness and genetic structure (Olmstead 1990) or about the influence of environmental traits on levels of genetic variation. Gitzendanner and Soltis (2000) analyze the values of genetic variation in rare species and highlight the importance of comparisons with data from their more widespread congeners (i.e. with phylogenetically close taxa).

Indeed, there are examples of approaches where phylogenetic information provided enhanced understanding of the evolutionary causes underlying the generation and maintenance of levels of isozyme variation (Warwick and Gottlieb 1985, Olmstead 1990). On the other side, environmental variables are often disregarded as a factor when interpreting genetic variability patterns, although they are a driving influence in diversification processes (Graham 1997) and, consequently, in the genetic structuring of species.

Previous surveys with *Androcymbium* (Pedrola-Monfort and Caujapé-Castells 1994, 1995, 1996; Caujapé-Castells 1995; Caujapé-Castells and Pedrola-Monfort 1997) showed that the isozymic variation of North African species is much higher than the estimates reported for other monocots or for species with similar geographic ranges (Hamrick and Godt 1989, 1996). Further, these studies concluded that most of this molecular variation is attributable to the intra-populational component. The paucity of available data of this kind for the species in South Africa (Caujapé-Castells et al. 1999a) has prevented a thorough comparison between two of the disjunct distribution areas of the genus and therefore hindered understanding how and why isozyme diversity varies in *Androcymbium*. A recent phylogenetic analysis based on cpDNA RFLPs (Caujapé-Castells et al. 1999b) results in a contrasting phylogenetic structure between North and South Africa; while the six species at the former area were strictly monophyletic, the species from South Africa distributed in three clades supported by high bootstrap values.

Both the striking environmental contrasts between North Africa/South Africa (and within South Africa) and the different phylogenetic structures of taxa in either area of distribution indirectly suggest that levels of genetic variation in *Androcymbium* might be influenced by environmental variables and phylogenetic affinities. Our objective in this work is twofold. First, to examine isozyme variation in a comprehensive sample of *Androcymbium* species in South Africa and to compare it with data for their congeners in North Africa. Second, to understand the relative influence of biological, phylogenetic and historical-environmental factors on the genetic isozymic variation in the two geographic distribution areas of this African disjunction.

Materials and methods

Sampling. A total of 32 populations (906 individuals) representing a thorough geographical sampling of 17 taxa of genus *Androcymbium* in South Africa were collected during August 1994 (Table 1; Fig. 1). The sampling scheme used for the South African species included in this work is similar to that employed for their congeners in North Africa (Pedrola-Monfort and Caujapé-Castells 1994, 1996) in terms of total number of populations (NP in Table 2) and individuals sampled (N in Table 2). The sampling was carried out along transects to maximize the chance of obtaining an accurate representation of populational variation. However, the number of sampled populations in South Africa is smaller because of the much narrower geographical ranges of *Androcymbium* species in this area of occurrence. These different patterns of space occupancy reflect the ecological contrasts between North and South Africa. In North Africa, characterized by a remarkable environmental and climatic homogeneity, there are six species with up to 15–20 populations in the more widespread *A. gramineum*, *A. rechingerii* and *A. wyssianum*. Because of these widespread ranges, the North African species had a high populational representation in our collection designs for previous surveys (Pedrola-Monfort and Caujapé-Castells 1996). In South Africa, where sharp edaphic and climatic heterogeneity is the rule, there are over 35 species known in most cases only from a few populations. Because of these narrow ranges of distribution and of the botanical underexploration of the Cape Region for Liliaceae (Milton et al. 1997), many of the South African species are represented in our sampling for this work by a single population.

Live specimens were carefully unearthed, assigned a code, put into paper bags and transported from South Africa to the Estació Internacional de Biologia Mediterrània-Jardí Botànic Marimurtra in Blanes (Spain), where they were planted at the greenhouses under homogeneous environmental

Table 1. Sampling details for the 17 South African *Androcymbium* taxa studied. The voucher references of these populations at the Jardí Botànic Marimurtra herbarium (JBMM) are cited in brackets after the population codes. These populations were collected by J. Pedrola-Monfort, J. Caujapé-Castells, J. Gibert, L. Llorens, X. Tebar and R. Echevarne in July–August 1994

Species	Population codes	Location
A. albanense subsp. *clanwilliamense* Pedrola-Monfort, Membrives & J.M. Monts.	1. ALBA-PK (JBMM 1386)	Pakhuispass
A. austrocapense U. Müll.-Doblies & D. Müll.-Doblies	2. AUST-GH (JBMM 1371)	Good Hope
	3. AUST-WP (JBMM 1370)	Whale's Point
A. bellum Schltr. & K. Krause	4. BELL-VI (JBMM 1378)	Vioolosdrift
A. burchellii subsp. *burchellii* Baker	5. BURC-HX (JBMM 1368)	Hexrivier
A. burchellii subsp. *pulchrum* Schinz	6. PULC-CA (JBMM 1385)	Calvinia
	7. PULC-NI (JBMM 1384)	Nieuwoudtville
A. capense (L.) K. Krause	8. CAPE-HO (JBMM 1647)	Hopefield
A. circinatum Baker	9. CIRC-NB (JBMM 1389)	Nababiep
	10. CIRC-SB (JBMM 1388)	Springbok
A. cuspidatum Baker	11. CUSP-CA (JBMM 1391)	Calvinia
	12. CUSP-MO (JBMM 1367)	Montagu
A. dregei C. Presl	13. DREG-PK (JBMM 1663)	Pakhuispass
A. eghimocymbion U. Müll.-Doblies & D. Müll.-Doblies	14. EGHI-CI (JBMM 1662)	Clanwilliam
	15. EGHI-PK (JBMM 1661)	Wuppertal
A. hantamense Engl.	16. HANT-CA (JBMM 1390)	Calvinia
A. henssenianum U. Müll.-Doblies & D. Müll.-Doblies	17. HENS-EK (JBMM 1658)	Eksteenfontein
A. huntleyi Pedrola-Monfort, Membrives, J.M. Monts. & Caujapé-Castells	18. HUNT-EK1 (JBMM 1660)	Eksteenfontein
	19. HUNT-EK3 (JBMM 1659)	Eksteenfontein
A. irroratum Baker	20. IRRO-EK (JBMM 1669)	Eksteenfontein
	21. IRRO-EK2 (JBMM 1667)	Eksteenfontein
	22. IRRO-EK6 (JBMM 1668)	Eksteenfontein
	23. IRRO-KW (JBMM 1664)	Kwagaskllof
	24. IRRO-VY (JBMM 1665)	Vanrhynsdorp
	25. IRRO-VP (JBMM 1387)	Vanrhynspass
	26. IRRO-KA (JBMM 1666)	Kamiesberg
A. poeltianum U. Müll.-Doblies & D. Müll.-Doblies	27. POEL-ST (JBMM 1657)	Steinkopf
	28. POEL-CO (JBMM 1377)	Concordia
	29. POEL-NB (JBMM 1376)	Nababiep
A. villosum U. Müll.-Doblies & D. Müll.-Doblies	30. VILL-ST (JBMM 1654)	Steinkopf
	31. VILL-EK (JBMM 1653)	Eksteenfontein
A. walteri Pedrola-Monfort, Membrives, J.M. Monts. & Caujapé-Castells	32. WALT-ST (JBMM 1651)	Steinkopf

conditions. These taxa exhibit an aneuploid series of $2n = 18$, 20 and 22 (Margelí et al. 1999, Montserrat et al. unpubl. data), whereas all the species in North Africa are diploid ($2n = 18$).

Reproductive traits. Reproductive data obtained from controlled crosses between plants grown under uniform temperature, humidity, light and substrate allowed us to infer the breeding system and the mode of reproduction of *Androcymbium* taxa (Membrives 2000). Whenever it was possible, all the reproductive biology experiments were made with a minimum of three individuals per popula-

Table 2. Results of reproductive biology experiments in the three kinds of artificial crossings described in the text as measured by the average number of seeds per capsule ± standard deviation (number of capsules studied in brackets). RS = Reproductive system (SC = self-compatible, PSI = preferentially self-incompatible, OS = obligate selfing)

Taxa	Vegetative reproduction (%)	Spontaneous self-compatibility	Obligate self-compatibility	Cross-pollination	RS
A. albanense[1]	37.5	64 ± 40 (10)	74 ± 40 (7)	86 ± 46 (5)	SC
A. austrocapence	19.2	58 ± 38 (38)	101 ± 39 (52)	81 ± 18 (10)	SC
A. bellum	72.2	[3 (1)]	4 ± 4 (5)	109 ± 60 (4)	PSI
A. burchellii[2]	1.6	[0 (2)]	3 ± 6 (5)	74 ± 63 (8)	PSI
A. burchellii[3]	2.2	1 ± 2 (4)	26 ± 39 (16)	98 ± 56 (8)	PSI
A. capense	21.3	166 ± 50 (5)	139 ± 73 (2)	148 ± 91 (11)	SC
A. circinatum	31.8	[0 (1)]	[0 (2)]	6 ± 8 (8)	PSI
A. cuspidatum	0	66 ± 28 (9)	73 ± 34 (14)	90 ± 21 (3)	SC
A. dregei	0	22 ± 17 (4)	22 ± 12 (7)	57 ± 0 (2)	SC
A. eghimocymbion	0	73 ± 65 (3)	130 ± 65 (6)	[–]	OS
A. hantamense	49.1	[0 (4)]	3 ± 4 (18)	2 ± 3 (5)	PSI
A. henssenianum	0	9 ± 9 (6)	25 ± 11 (7)	51 ± 21 (8)	SC
A. huntleyi	31.3	73 ± 47 (2)	112 ± 57 (3)	100 (1)	SC
A. irroratum	2.3	199 ± 142 (10)	166 ± 102 (18)	207 ± 107 (18)	SC
A. poeltianum	45.7	145 ± 70 (5)	121 ± 41 (3)	101 ± 64 (8)	SC
A. villosum	16.2	[0 (1)]	8 ± 7 (3)	73 ± 69 (5)	PSI
A. walteri	64.3	[0 (1)]	16 ± 4 (3)	157 ± 105 (14)	PSI

subsp. *clanwilliamense*[1]; subsp. *burchellii*[2]; subsp. *pulchrum*[3]

tion. These experiments were aimed at examining the degree of: (1) spontaneous self-compatibility (flowers were bagged and no further manipulation was carried out), (2) obligate self-compatibility (stigmas were artificially self-pollinated with pollen from the same flower), and (3) cross-pollination (stigmas were artificially pollinated with pollen from a different individual). The number of seeds obtained from these three kinds of artificial crossings are given in Table 2. Inferences of the breeding system (self-compatibility or self-incompatibility) were based on a Student-t test that compared the average number of seeds per capsule in the reproductive experiments of obligate self-compatibility with the average number of seeds obtained in the cross-pollination experiments (Membrives 2000). If the number of seeds from the cross-pollination experiments was significantly higher than that from obligate self-compatibility experiments then the breeding system was inferred to be preferentially self-incompatible. When the differences in seed number between the two kinds of experiments were not significant, then we assumed that the breeding system was self-compatible.

The mode of reproduction (sexual and asexual, or only sexual) was inferred from the percentages of vegetative reproduction observed in the greenhouse for each species. These percentages correspond to observations carried out the same year and were estimated through the quotient between the number of flowering individuals that gave rise to vegetative subdivisions (i.e. bulbils budding off the mother bulb) and the total number of flowering individuals. We only reckoned the flowering individuals in order to ensure that all the specimens considered were adults. The mode of reproduction of the species was defined as "sexual and asexual" when this percentage was higher than 5% per year and as "only sexual" otherwise. "Only asexual" does not seem an option in nature, since all studied species produced seeds in the artificial pollination experiments described above.

Electrophoretic analyses. Extracts were made from tips of young leaves, except for those involved in the allelic homologies of ADH, where we used seeds previously soaked in water (Gottlieb 1981). The analytical protocols are those in Pedrola-Monfort and Caujapé-Castells (1996) with

a few minor modifications in the amounts of substrate in the staining solutions. Three buffer systems (Shields et al. 1983) were used to resolve eight enzyme systems. Histidine pH 7.0 resolved aconitase (ACO, E.C. 4.2.1.3), phosphoglucomutase (PGM, E.C. 5.4.2.2), phosphoglucose isomerase (PGI, E.C. 5.3.1.9), malate dehydrogenase (MDH, E.C. 1.1.1.37), and alcohol dehydrogenase (ADH, E.C. 1.1.1.1). Tris-Citrate pH 8.2 resolved malic enzyme (ME, E.C. 1.1.1.40) and glutamate dehydrogenase (GDH, E.C. 1.4.1.2). Finally, Tris-Citrate pH 5.7 resolved phosphogluconic dehydrogenase (6PGD, E.C. 1.1.1.44). The enzyme patterns were analyzed on an allelic base. For each enzyme, gene loci and isozymes were labelled 1, 2, 3… and, for each locus, alleles were labelled a, b, c,…, beginning with the most anodally migrating bands. Two enzymes (GDH and ME) were resolved using acrylamide gels as described in Shields et al. (1983). We did not carry out a program of crosses to ascertain the genetic basis of the banding patterns because of the time required (3–4 yr) for plants grown from seed to develop leaves suitable for electrophoresis. Intra-population, inter-population and inter-specific verifications of enzyme mobilities were determined through side-by-side comparisons of allelic variants on the same gel.

Data analysis. The number of alleles per locus (A), percentage of polymorphic loci (P), observed and expected heterozygosity (Ho and He), and genetic identities (Nei 1978) were calculated using BIOSYS-1 Version 1.7 (Swofford and Selander 1989). All calculations were made at the species (As, Ps, Hos and Hes), and population (Ap, Pp, Hop and Hep) levels from genotype data corresponding to each locus.

The proportion of total variation explained by the inter-populational component (Gst, Nei 1973) and related population-structure statistics were calculated over all loci using GeneStat-PC 3.31 (Lewis and Whitkus 1993). The effective number of alleles (Ae) was calculated following Kimura and Crow (1964). The fixation index (F, which is a function of observed and expected heterozygosities) was calculated following Wright (1951). Values of these parameters for species in North Africa were taken directly from Pedrola-Monfort and Caujapé-Castells (1996) or, for the number of alleles per locus (A) and effective number of alleles (Ae), calculated from data from that study.

A total of five zones of enzymatic activity were detected for 6PGD and prospectively interpreted as duplications, and another putative duplication was interpreted for GDH (see below for further details). Since we could not determine whether the observed variation in the number of putative loci across species for these two enzymes was related to different degrees of gene silencing or derived from a different number of loci, they were not considered to calculate genetic identities. We built a UPGMA similarity tree by using the ten remaining loci. Nei's (1978) genetic distances were calibrated against the age of the common ancestor of the species of North Africa as estimated from cpDNA restriction fragment data (Caujapé-Castells et al. 2001). This calibration was used to calculate divergence times at 9 nodes representing major diversification events on the UPGMA tree (Fig. 2).

Pearson correlations between every pairwise combination of isozymic (Pp, Hep, and Gst), reproductive (mode of reproduction and breeding system) and geographical (latitudinal distribution) variables in South African *Androcymbium* taxa were calculated using the statistical package SPSS for windows Version 6.1.2 (1995). We coded the mode of reproduction (0: only sexual; 1: sexual and asexual) and the breeding system (0: self-compatible species; 1: self-incompatible species) to calculate the correlations with the isozyme variation parameters. Thirteen edaphic parameters (pH, water retention capacity, salinity, % of total carbonates, % of organic material, amount of mineral nitrogen, amount of assimilable phosphor, amount of assimilable potassium, cathionic interchange capacity, %sand, %lime, %clay, and soil texture) were taken from population soil analyses (Membrives et al. 2001). We calculated edaphic distances using the option 'taxonomic distance' in NTSYS-pc after standardizing the values of the 13 edaphic parameters. The value and significance of the correlation between Nei's (1978) isozyme distance matrix and the edaphic distance matrix was evaluated through Mantel tests (Mantel 1967) using NTSYS-pc (Rohlf 1988).

Results

Electrophoretic profiles. The use of eight enzymes allowed the resolution of 16 putative isozyme loci (Appendix). Banding patterns associated with heterozygous individuals were

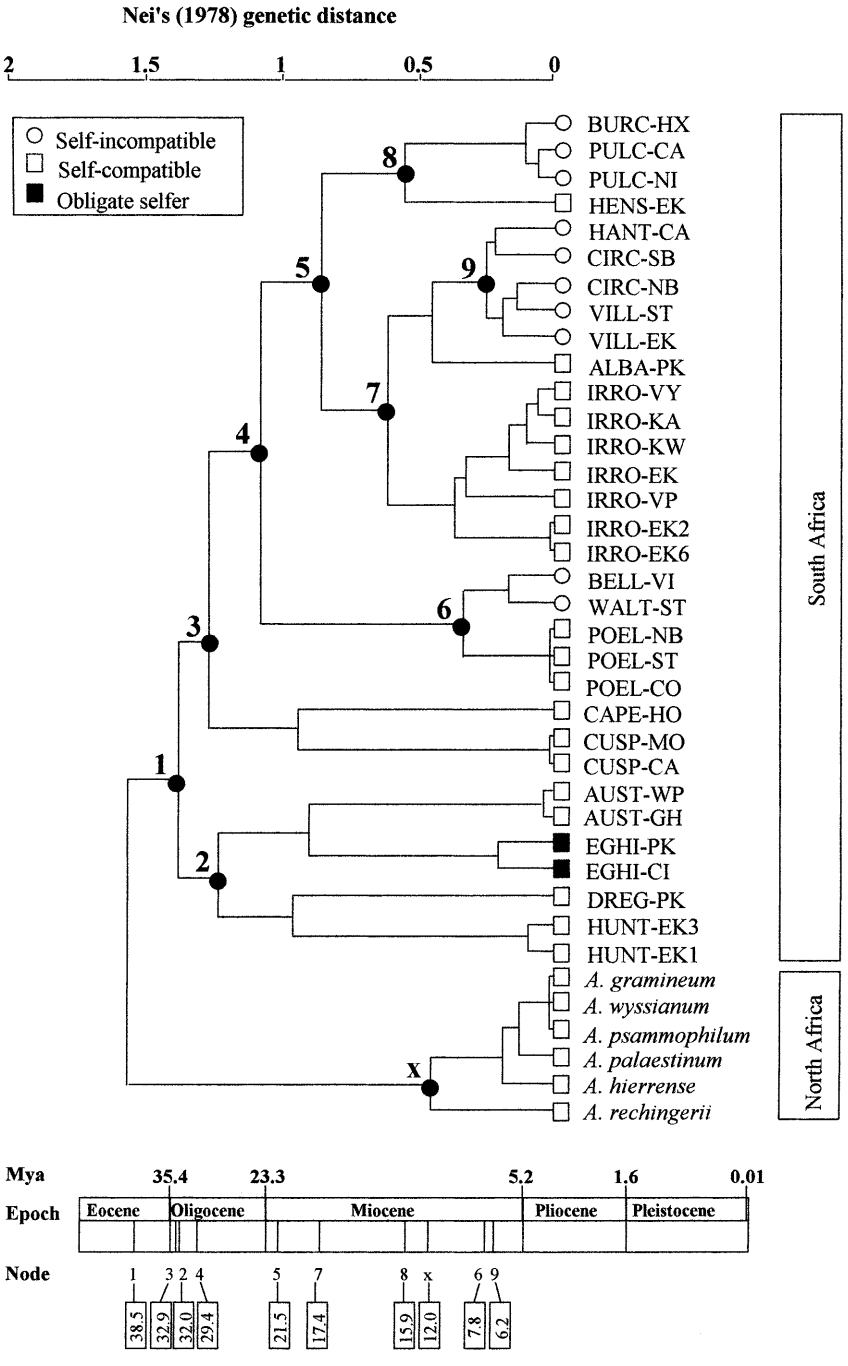

Fig. 2. UPGMA tree of Nei's (1978) genetic distances between the 32 populations in South Africa analyzed in this study and the six North African species. Divergence times for the nodes labelled 1 to 9 are given in mya and mapped onto the chronology (below). The dating of the node labelled 'x' corresponds to 12 mya (Caujapé-Castells et al. 2001) and was used to calibrate the isozyme molecular clock (see text for explanation). Species codes are those in Table 1

consistent with the expected quaternary structures in diploid plants (Gottlieb 1981, 1982) for all enzymes analyzed except for GDH. This enzyme showed seven different bands in all the surveyed individuals of *A. austrocapense*, *A. capense* and *A. irroratum*. While these banding patterns were in agreement with the expected hexameric structure for heterozygous individuals (Cammerts and Jacobs 1983), the central bands were not the most intense (as would be expected with codominant inheritance), and separation between them was not symmetrical.

Electrophoresis of ten seedlings obtained from artificial self-fertilization from the same individual of *A. irroratum* revealed that the seven bands had been inherited in all cases. We interpreted these results as monomorphic heterozygosity derived from a single duplication of GDH (following Crawford et al. 1990). The progressive fading of band intensity towards the anode was explained by hypothesizing a lower activity of the duplicate enzymes. The total number of alleles detected over these 16 loci in South African taxa (128 alleles) was far higher than that found in previous works with their North African congeners (Pedrola-Monfort and Caujapé-Castells 1996) for the same loci (82 alleles).

Intra-populational variation. Except for *A. henssenianum* (that is monomorphic for the 16 loci studied), all *Androcymbium* species in South Africa show isozyme polymorphism in one or more loci. The average number of alleles per locus per population (Ap) ranged from 1.0 in *A. henssenianum* to 2.08 in *A. burchellii* subsp. *pulchrum*, with an average of 1.44 (Table 3). The effective number of alleles per locus per population (Aep) varied between 1.00 for *A. henssenianum* and 1.33 for *A. burchellii* subsp. *pulchrum* and *A. villosum*, with an average over species of 1.15. The percentage of polymorphic loci per species (Ps) was 37.32% (ranging from 0% in *A. henssenianum* to 80.0% in *A. irroratum*). The percentage of polymorphic loci per population (Pp) was 29.98% and ranged from 0% in *A. henssenianum* to 69.23% in *A. walteri*. The average observed heterozygosity per species (Hos) was 0.121 (ranging from 0.000 in *A. henssenianum* to 0.259 in *A. burchellii* subsp. *pulchrum*). The average observed heterozygosity per population (Hop) was 0.121 (0.000 for both *A. huntleyi* and *A. henssenianum*, and 0.270 for *A. burchellii* subsp. *pulchrum*). The average expected heterozygosity per species (Hes) was 0.145 (ranging from 0.000 in *A. henssenianum* to 0.308 in *A. villosum*). The average expected heterozygosity per population (Hep) was 0.121 (0.000 for *A. henssenianum* to 0.250 for *A. villosum*).

Wright's (1951) fixation index (F) gives an estimate of the degree of inbreeding, with $F = 0$ indicating random mating. Negative values of F indicate heterozygote excess and are found in species with high percentages of vegetative reproduction. Positive values of F indicate homozygote excess and are generally found in self-compatible species or in those having a population sub-structuring leading to an increase in assortive mating (Levin and Kerster 1974). Values of this parameter for *Androcymbium* species in South Africa (Table 3) ranged from $F = 1$ in *A. huntleyi* (where no heterozygotes were observed) to $F = -0.97$ in *A. poeltianum*, with a mean value over species of $F = -0.04$. Unexpectedly, most self-compatible species showed negative F values.

Inter-populational apportionment of genetic variation. Given that the species in South Africa are very narrow endemics, just one population could be sampled for many of them. Consequently, we could only characterize the inter-populational distribution of variability in the nine taxa where two or more populations were sampled (Table 4). According to our estimates of total diversity (Ht), the least variable species was *A. henssenianum* ($Ht = 0.000$), whereas *A. villosum* ($Ht = 0.320$) was the most variable. The mean value of Ht for all species in South Africa analyzed was 0.183. The amount of isozyme variability attributable to the inter-population component in South African species ranged from 0% in *A. poeltianum* to 78.2% in *A. irroratum*. The mean value of Gst for taxa in South Africa was 30.8%.

Genetic identity. Average genetic identity (Nei 1978) for conspecific populations of *Androcymbium* in South Africa (Table 5) varied from 0.715 for *A. irroratum* to 1.000 for *A. poeltianum*, with an average over species of 0.083. The genetic identity value between subspecies *A. burchellii* subsp. *burchellii* and *A. burchellii* subsp. *pulchrum* was $I = 0.956$. Interspecific genetic identity varied from a surprisingly low $I = 0.003$ between *A. dregei*/*A. capense* and *A. eghimocymbion*/*A. capense* to $I = 0.843$ between *A. hantamense*/*A. circinatum*.

Table 3. Summary of basic isozymic variation parameters in the 17 taxa of *Androcymbium* in South Africa and in the six taxa in North Africa (after Pedrola-Monfort and Caujapé-Castells 1994, 1996). NL: Number of loci analyzed; NP: number of populations studied; N: mean sample size; Ap: number of alleles per locus; Aep: effective number of alleles per populations; Ps and Pp: mean percentage of loci polymorphic per species and per population (without criterion); Hos and Hop: observed heterozygosity per species and per population; Hes and Hep: expected heterozygosity per species and per population; F: Wright's fixation index. The F value could not be calculated in *A. henssenianum* owing to the null values of both Hos and Hes in this species

Region/Breeding System/Species	NL	NP	N	Ap	Aep	Ps	Pp	Hos	Hop	Hes	Hep	F
South Africa												
Self-incompatible taxa												
A. bellum	13	1	73	2.00	1.22	38.46	38.46	0.182	0.182	0.180	0.180	−0.01
A. burchellii subsp. *burchellii*	12	1	27	1.83	1.20	50.00	50.00	0.163	0.163	0.170	0.170	0.04
A. burchellii subsp. *pulchrum*	12	2	63	2.08	1.33	75.00	66.67	0.259	0.270	0.286	0.249	−0.08
A. circinatum	12	2	73	1.79	1.31	66.67	50.00	0.199	0.204	0.232	0.238	0.14
A. hantamense	13	1	38	1.46	1.18	38.46	38.46	0.119	0.119	0.149	0.149	0.20
A. villosum	12	2	56	1.79	1.33	66.67	50.00	0.210	0.217	0.308	0.250	0.13
A. walteri	13	1	26	1.92	1.29	69.23	69.23	0.228	0.228	0.225	0.225	−0.01
Self-compatible taxa												
A. albanense subsp. *clanwilliamense*	15	1	95	1.27	1.12	20.00	20.00	0.119	0.119	0.111	0.111	−0.07
A. austrocapense	15	2	64	1.17	1.09	20.00	16.65	0.132	0.132	0.089	0.085	−0.55
A. capense	13	1	53	1.38	1.10	30.77	30.77	0.154	0.154	0.092	0.092	−0.67
A. cuspidatum	14	2	48	1.14	1.05	14.29	14.29	0.051	0.043	0.060	0.048	0.10
A. dregei	14	1	27	1.14	1.06	14.29	14.29	0.005	0.005	0.059	0.059	0.92
A. eghimocymbion	14	2	30	1.14	1.04	21.43	7.14	0.071	0.071	0.116	0.041	−0.73
A. henssenianum	13	1	54	1.00	1.00	0.00	0.00	0.000	0.000	0.000	0.000	–
A. huntleyi	14	2	12	1.11	1.05	21.43	10.72	0.000	0.000	0.062	0.051	1.00
A. irroratum	15	7	108	1.17	1.08	80.00	13.33	0.083	0.073	0.279	0.068	−0.07
A. poeltianum	13	3	22	1.08	1.04	7.69	7.69	0.077	0.077	0.039	0.039	−0.97
Average self-incompatible			51	1.84	1.27	57.78	51.83	0.194	0.197	0.221	0.209	0.06
Average self-compatible			51	1.16	1.06	22.99	13.49	0.069	0.067	0.091	0.059	−0.12
Average South Africa			51	1.44	1.15	37.32	29.28	0.121	0.121	0.145	0.121	−0.04
North Africa												
Mainland area												
A. gramineum	10	15	300	1.80	1.24	100.0	60.7	0.153	0.165	0.216	0.193	0.27
A. palaestinum	10	2	15	1.90	1.39	80.0	70.0	0.192	0.273	0.241	0.283	0.20
A. rechingerii	10	1	30	2.00	1.49	80.0	80.0	0.278	0.278	0.327	0.327	0.15
A. wyssianum	10	10	87	2.00	1.32	70.0	73.3	0.203	0.232	0.238	0.243	0.15
Canary Island taxa												
A. hierrense	10	3	65	1.10	1.06	20	13.3	0.105	0.107	0.055	0.058	−0.91
A. psammophilum	10	2	65	1.10	1.06	10	15.0	0.100	0.100	0.053	0.053	−0.89
Average mainland North Africa			108	1.93	1.36	82.5	71.0	0.207	0.237	0.256	0.262	0.19
Average Canaries			28	1.10	1.06	15	14.2	0.103	0.104	0.054	0.056	−0.45
Average North Africa			94	1.85	1.32	60	52.0	0.172	0.193	0.188	0.193	−0.02

Table 4. Summary of population genetic structure statistics in taxa of *Androcymbium* in South and North Africa Ht: total genetic diversity; Hs: population-level genetic diversity; Dst: population differentiation; Gst: genetic differentiation between populations (Dst and Gst are given only for species where more than one population was available)

Region/Breeding system/Species	Ht	Hs	Dst	Gst
South Africa				
Preferentially self-incompatible taxa				
A. bellum	0.180	0.180	–	–
A. burchellii	0.170	0.170	–	–
A. circinatum	0.301	0.232	0.070	23.3
A. hantamense	0.149	0.149	–	–
A. burchellii subsp. *pulchrum*	0.288	0.244	0.044	15.3
A. villosum	0.320	0.243	0.077	24.1
A. walteri	0.225	0.225	–	–
Self-compatible taxa				
A. albanense subsp. *clanwilliamense*	0.111	0.111	–	–
A. austrocapense	0.092	0.083	0.009	9.8
A. capense	0.092	0.092	–	–
A. cuspidatum	0.058	0.047	0.010	17.2
A. dregei	0.059	0.059	–	–
A. eghimocymbion	0.125	0.039	0.086	68.8
A. henssenianum	0.000	0.000	–	–
A. huntleyi	0.076	0.045	0.031	40.8
A. irroratum	0.298	0.065	0.233	78.2
A. poeltianum	0.038	0.038	0.000	0
Average preferentially self-incompatible	0.234	0.206	0.064	20.9
Average self-compatible	0.095	0.058	0.062	35.8
Average South Africa	0.183	0.143	0.073	30.8
North Africa				
Mainland taxa				
A. gramineum	0.246	0.186	0.060	24.4
A. palaestinum	0.300	0.248	0.052	17.2
A. rechingerii	0.327	0.327	–	–
A. wyssianum	0.248	0.219	0.029	11.5
Canary Island taxa				
A. hierrense	0.057	0.056	0.001	0.9
A. psammophilum	0.052	0.052	0.000	0
Average mainland North Africa	0.280	0.163	0.035	13.3
Average Canaries	0.055	0.054	0.001	0.5
Average North Africa	0.205	0.163	0.035	10.8

The congeneric average for the species in South Africa was $I = 0.364$.

A time scale was added to the UPGMA tree (Fig. 2) assuming that the ancestor of the species in North Africa corresponds to 12 mya (Caujapé-Castells et al. 2001). Provided this is a reasonable divergence time reference, a Nei's (1978) genetic distance of 0.100 equals 2.52 mya. This result is similar to that estimated for some vertebrate groups (Wayne and O'Brien 1987). Because the taxa of *Androcymbium* are closely related and exhibit the same growth habit, it seems unlikely that the variability in the rate of protein evolution

Table 5. Genetic identities (Nei 1978) between *Androcymbium* taxa in South Africa. Average intra-specific identities for the taxa where we sampled more than one population are given in the diagonal

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. *A. burchellii*[1]	–																
2. *A. capense*	0.275	–															
3. *A. austrocapense*	0.128	0.129	0.982														
4. *A. burchellii*[2]	0.956	0.252	0.123	–													
5. *A. hantamense*	0.655	0.242	0.235	0.640	–												
6. *A. albanense*[3]	0.544	0.245	0.230	0.543	0.655	–											
7. *A. dregei*	0.242	0.003	0.165	0.286	0.301	0.271	–										
8. *A. circinatum*	0.561	0.233	0.241	0.579	0.843	0.575	0.309	0.824									
9. *A. villosum*	0.548	0.221	0.260	0.546	0.784	0.541	0.308	0.820	0.804								
10. *A. eghimocymbion*	0.247	0.003	0.411	0.338	0.424	0.310	0.489	0.416	0.406	0.823							
11. *A. cuspidatum*	0.372	0.373	0.077	0.356	0.342	0.294	0.098	0.346	0.377	0.107	0.978						
12. *A. huntleyi*	0.274	0.177	0.302	0.302	0.435	0.298	0.474	0.461	0.422	0.319	0.193	0.941					
13. *A. henssenianum*	0.551	0.183	0.114	0.578	0.430	0.519	0.186	0.392	0.339	0.158	0.278	0.109	–				
14. *A. bellum*	0.301	0.374	0.248	0.276	0.574	0.451	0.135	0.552	0.509	0.270	0.408	0.340	0.299	–			
15. *A. poeltianum*	0.365	0.275	0.264	0.346	0.491	0.415	0.093	0.552	0.438	0.285	0.290	0.284	0.364	0.773	1.000		
16. *A. walteri*	0.396	0.350	0.194	0.378	0.537	0.420	0.264	0.622	0.468	0.289	0.301	0.358	0.303	0.842	0.742	–	
17. *A. irroratum*	0.444	0.205	0.140	0.512	0.548	0.372	0.287	0.486	0.485	0.285	0.308	0.259	0.218	0.284	0.171	0.294	0.715

1: subsp. *burchellii*; 2: subsp. *pulchrum*; 3: subsp. *clanwilliamense*

is substantial. According to our calibration of the isozyme molecular clock of *Androcymbium* (Fig. 2) the first species diversifications occurred in South Africa (nodes 2 and 3 in Fig. 2) were 32.0 mya and 32.9 mya, respectively (early Oligocene), and most of the diversification events of self-compatible species in South Africa occurred in the early and mid-Miocene. All self-incompatible species (*A. bellum*, *A. burchellii* subsp. *burchellii*, *A. burchellii* subsp. *pulchrum*, *A. circinatum*, *A. hantamense*, *A. villosum* and *A. walteri*) diversified in the late Miocene (nodes 6, 8 and 9 in Fig. 2), coincident with inter-populational diversification in South Africa and incipient speciation in North Africa.

Genetic variation, reproductive traits, edaphic parameters and geographical distribution. We found no significant correlation between genetic diversity statistics (Pp, Hep and Gst) and mode of reproduction in *Androcymbium* species in South Africa (Table 6). By contrast, breeding systems (self-compatible vs. self-incompatible) showed significant correlations with Hep (90.9%) and Pp (89.5%). Correlations between Gst and breeding systems (Table 6) were non-significant. Mantel tests failed to detect correlation between the isozymic and the edaphic distance matrices ($r = 0.029$; $p = 0.615$). Latitudinal distribution was significantly correlated with genetic variation only for self-compatible populations from South Africa (59.9% for Pp and 52.3% for Hep). In general, self-incompatible species distributed in the north of South Africa show lower genetic diversity levels than those in the south (Fig. 3).

Whereas all species in North Africa are self-compatible, the breeding system is variable in South African taxa (Table 2). Because of the high correlation between breeding system and genetic variation levels in *Androcymbium* (Table 6), our comparisons between the two disjunct regions were based on self-compatible species alone. The values of genetic variation parameters in species from North Africa were significantly higher than those in species in South Africa ($t = 3.90$, $p < 0.01$ for Pp; $t = 3.49$, $p < 0.01$ for Hep). No significant differences were found at this level between Gst values.

Discussion

The amount and distribution of genetic diversity for a given species depend on the interaction between the biological and ecological factors that determine the extent of gene flow in space and the historical and environmental factors that affect evolutionary dynamics. Based on present knowledge of the biological features that characterize *Androcymbium*, the available historical information on South Africa and a recent phylogenetic hypothesis based on cpDNA RFLPs (Caujapé-Castells et al. 1999b), we will address two interrelated issues. First, whether we can predict the levels of genetic diversity in *Androcymbium*. And second, which factors seem to be most

Table 6. Correlations between population genetic variation statistics and biological and geographical traits for taxa of *Androcymbium* in South Africa

Biological and geographical traits	Genetic diversity statistics		
	Pp	Hep	Gst
Mode of reproduction	r = 0.222	r = 0.312	r = 0.379
Breeding system	r = 0.895***	r = 0.909***	r = 0.281
Latitudinal distribution (all populations)	r = 0.015	r = 0.042	–
Latitudinal distribution (self-incompatible species)	r = 0.359	r = 0.105	–
Latitudinal distribution (self-compatible species)	r = 0.599**	r = 0.532*	–

*p < 0.05; **p < 0.01; ***p < 0.001

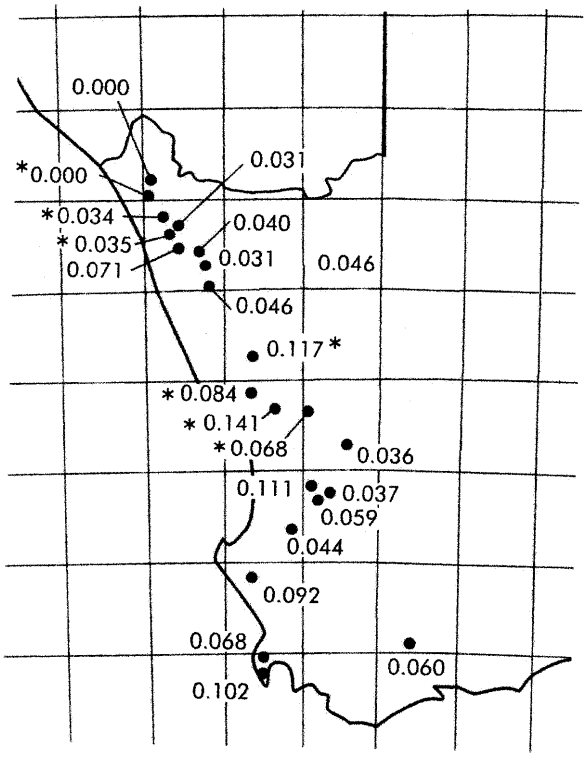

Fig. 3. Geographical distribution of the values of expected heterozygosity for the sampled populations of self-compatible *Androcymbium* species in South Africa. The correlation between the latitudinal distribution of taxa and genetic variation is significant, as shown in Table 6. Asterisks signal the populations of *A. irroratum*

influential on the amounts and distribution of genetic variation in the genus.

Reproductive biology and genetic variation. Isozyme variation in the taxa of *Androcymbium* in South Africa are dramatically influenced by the breeding system. Comparison with reports by Gottlieb (1981), Loveless and Hamrick (1984) and Hamrick and Godt (1989) reveals that preferentially self-incompatible *Androcymbium* species display high levels of variation at the populational level (Table 3), either considering this breeding system alone or jointly with geographical distribution (Hamrick and Godt 1997). The only exceptions are *A. bellum* (Ps = 38.46, Hes = 0.180) and *A. hantamense* (Ps = 38.46, Hes = 0.149) where the low genetic diversity levels could be explained by the high incidence of vegetative reproduction observed in the greenhouse (Table 2). However, this hypothesis is not reflected by high negative values of F (Table 3).

The proportion of variation explained by the inter-populational component in preferentially self-incompatible *Androcymbium* in South Africa (Gst = 20.9% in Table 4) is similar to the expected value for outcrossing endemics (Gst = 17.9%) according to Hamrick and Godt (1996). Comparatively high inter-population differentiation (Table 4) and low intra-specific genetic identities (Table 5) in *A. circinatum* and *A. villosum* suggest two possible diversification scenarios. First, and ancient interpopulational divergence. Second, a rapid fixation of different alleles in the populations as a consequence of bottleneck situations. The two possibilities seem likely in the face of the ecological diversity of the landscape in South Africa, which could have fostered the selection of different alleles depending on the geographical location of the populations. However, interpreting a rapid fixation and loss of alleles seems difficult unless the plants were highly selfing. Given that both species are preferentially self-incompatible (Table 2), the hypothesis of an ancient origin seems more probable.

The values of the basic statistics of isozymic variation for self-compatible *Androcymbium* species in South Africa (Table 3) are lower than those reported in the literature for species with mixed-animal breeding system (Hamrick and Godt 1996). When we consider this breeding system and geographic distribution jointly, our estimates (Ps = 22.9%, Hes = 0.091) are lower than those reported by Hamrick and Godt (1997) for endemics with mixed-animal pollination (Ps = 40.5%, Hes = 0.100). At the lowest extreme, *A. henssenianum* is known only by one population (Müller-Doblies and Müller-Doblies 1984) and is monomorphic for all analyzed loci. Although isozyme evidence indicates that this species probably arose via a founder event, genetic identity and morphology provide no

clues to hypothesize its probable origin. The fact that Nei's (1978) identities involving this species are very low relative to the identities between other species pairs (Table 5) does suggest a scenario of prolonged isolation and predominant action of drift. *Androcymbium henssenianum* has been viewed as related to *A. huntleyi* on morphological grounds (Pedrola-Monfort et al. 2001). However, our isozymic results do not seem to reflect this hypothesized relationship, since the genetic identity value between these two species (0.109) is one of the lowest observed within the genus (Table 5). Admittedly, these results might be biased by their being based on a few populations of each species, but only one population is known for *A. hensenianum* and two for *A. huntleyi* (Pedrola-Monfort et al. 1999).

Unlike their preferentially self-incompatible congeners, self-compatible species do not display similar levels of population differentiation as measured by Gst values (Table 4). Three of them (*A. austrocapense*, *A. cuspidatum* and *A. poeltianum*) show Gst values (0.098, 0.172 and 0.000, respectively) lower than those expected for endemic, mixed-animal pollinated species (Gst = 0.174 [Hamrick and Godt 1997]). This result and the high values of their intra-specific identities (Table 5) probably indicate recent population splitting. By contrast, other self-compatible species (*A. eghimocymbion*, *A. huntleyi* and *A. irroratum*) exhibit much higher Gst values (0.688, 0.408 and 0.780, respectively). The high Gst values in these species could have been caused by bottleneck situations and rapid fixation of different alleles in different populations. Yet, presently available evidence does not allow us to rule out that the high intra-specific differentiation could be caused by an ancient divergence. In *A. irroratum*, the high geographical isolation among populations might have added considerably to the dramatic inter-population differentiation. The very low average intra-specific genetic identity in *A. irroratum* (Table 5) and the conspicuous morphological differences among populations (Membrives 2000) do suggest a deeper taxonomic revision of this species.

Overall, the breeding system seems to be the best predictor of isozyme variation levels for taxa of *Androcymbium* in South Africa (Table 6) because it exhibits a correlation of roughly 0.900 with both genetic diversity (Hep) and the percentage of polymorphic loci (Pp). The average values of these two parameters for South African *Androcymbium* are higher than those reported by Hamrick and Godt (1989, 1996) and Olmstead (1990). At odds with the general trends revealed by Hamrick and Godt's (1989) analyses, the correlation between breeding system and Gst is not significant in *Androcymbium* (Table 6). Three feasible reasons for this disagreement could be (1) a bias due to the small number of populations examined, (2) a high degree of vegetative reproduction and assortive mating, and (3) different divergence times between populations depending on lineage circumscription.

Phylogenetic relationships and genetic variation. The accuracy of predictions concerning the structure of genetic variation is likely to increase when studies involve monophyletic groups (Loveless and Hamrick 1984, Warwick and Gottlieb 1985, Olmstead 1990, Gitzendanner and Soltis 2000). The only comprehensive phylogenetic hypothesis on *Androcymbium* available to date is based on parsimony analyses of cpDNA RFLPs (Caujapé-Castells et al. 1999b), and reveals three strongly supported monophyletic groups for the taxa in South Africa (Fig. 4). This topology disagrees with the UPGMA similarity tree based on Nei's (1978) genetic distance for isozymes (Fig. 2) in two aspects: first, in the close relatedness of *A. austrocapense* with taxa in North Africa in the cpDNA phylogeny (Fig. 4) vs. their separation in the similarity tree (Fig. 2); and second, in the conspicuous separation between *A. bellum* and the clade formed by *A. cuspidatum*, *A. poeltianum* and *A. walteri* in the cpDNA phylogeny (Fig. 4) vs. the closeness among these species in the similarity tree (Fig. 2). These contrasts are

likely attributable to the unequal mutation rates associated with either kind of molecular marker and to the different epistemological assumptions underlying the data analyses.

The only two studies known by us that compare levels of isozyme variation in monophyletic assemblages of species display contrasting results. Whereas Warwick and Gottlieb (1985) found that phylogenetically close species of *Layia* tend to have similar levels of genetic variation, Olmstead (1990) reports no correlation between levels of genetic variation and phylogenetic closeness in the *Scutellaria angustifolia* complex. The joint examination of the position of the taxa in the phylogenetic tree (Fig. 4) and the basic descriptors of genetic variation (Table 3) reveals that there is no relationship between phylogenetic relatedness and genetic variation levels in *Androcymbium*. By contrast, there is a remarkable correlation between genetic variation levels and reproductive features. The value of expected heterozygosity in self-incompatible *A. walteri* (Hep = 0.225) is closely similar to those of self-incompatible taxa in other clades such as *A. burchellii* subsp. *pulchrum* (Hep = 0.249), or *A. circinatum* (Hep = 0.238) (Fig. 4). An analogous situation holds for self-compatible taxa, where the values of expected heterozygosity are also consistently lower than those of their self-incompatible congeners. For instance, the expected heterozygosity of *A. austrocapense* (Hep = 0.085) is similar to that of *A. irroratum* (Hep = 0.068) or *A. albanense* subsp. *clanwilliamense* (Hep = 0.111), despite their different position in the phylogeny of Fig. 4.

Environmental factors, geographical disjunction and genetic variation. The relationship between genetic variation and environmental information is especially relevant in South Africa, where the hypothesis that environmental heterogeneity promoted species richness at a regional scale has strong support (Richerson and Lum 1980, Shmida and Wilson 1985, Linder 1985, Brown 1988, Diamond 1988, Williamson 1988, Cornell et al. 1997, Cowling et al. 1997). The scenario of conspicuous intraspecific morphological and isozymic differentiation in South African *Androcymbium* suggests speciation through environmental selection, as Linder (1985) construed for the Cape Flora. Thus, it is important to analyze the influence of environmental parameters

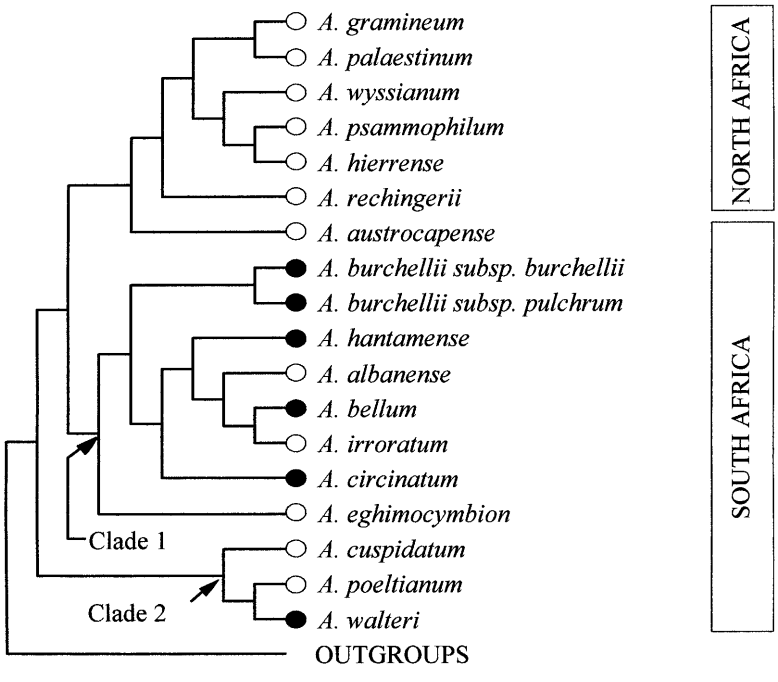

Fig. 4. Simplified clade topology of the section of the cpDNA RFLPs tree (Caujapé-Castells et al. 1999b) containing South African *Androcymbium* taxa. Black circles: preferentially self-incompatible taxa; Empty circles: self-compatible taxa

(edaphic and climatic) on the genus' speciation dynamics.

Although absence of significant correlation between edaphic variables and genetic distance argues against an influence of soil heterogeneity on genetic differentiation in South Africa, we do note a general trend of diminishing levels of isozymic variation as aridity increases towards the North for the self-compatible species (Fig. 3; Table 6). This trend is well illustrated with *A. irroratum*, whose three populations (IRRO-EK, IRRO-EK2 and IRRO-EK6) from the north of South Africa exhibit much lower values of expected heterozygosity than the four populations (IRRO-KA, IRRO-KW, IRRO-VP and IRRO-VY) from the south (Fig. 3). If other floristic rarities with a similar vicariant distribution in South Africa fit a similar pattern, we would expect that aridity could be a good predictor of genetic variation levels. Remarkably, Membrives et al. (2001) found that the edaphic characteristics associated with aridity (i.e. cathionic interchange capacity, water retention capacity, and percentage of clay) correlate with morphological leaf variability in South African *Androcymbium*. On the whole, the scenario of conspicuous intra-specific morphological and isozymic differentiation in South African *Androcymbium* suggests speciation through environmental selection, as Linder (1985) construed for the Cape Flora. A similar example of dependence of levels of genetic variation on environmental conditions is reported for Californian populations of *Chamaecyparis lawsoniana* (Millar and Marshall 1991), where higher variability indices in coastal populations than in interior populations are attributed to variable selection driven by the ecological differences between the two areas.

The consideration of the North-South disjunction of *Androcymbium* in the light of available biological data brings out four elements of contrast: (1) a much higher species diversity but narrower geographical species ranges in South Africa than in North Africa, (2) conspicuous inter-specific morphological, karyological and isozymic differences only in South Africa, (3) higher within population genetic variation levels in North Africa, and (4) higher total number of alleles in South Africa.

By virtue of their direct impact on distribution ranges of taxa, historical-environmental factors could help explain the sequence of events that may have influenced diversification processes (Graham 1997). Higher genetic variation patterns in terms of proportion of polymorphic loci, total number of alleles and observed expected heterozygosity are commonly associated with widespread geographic distributions (Loveless and Hamrick 1984; Hamrick and Godt 1989, 1997). Due to the numerous exceptions to this generalization, Gitzendanner and Soltis (2000) recommend to compare genetic variation levels and geographical distributions within congeneric species. Higher genetic variation levels in mainland North African *Androcymbium* taxa than in their South African congeners could be a consequence of their more widespread geographic distributions. Yet, the levels of isozymic variation in North African *Androcymbium* (Pedrola-Monfort and Caujapé-Castells 1994, 1996) rank much higher than expected for mixed-animal species with either a narrow ($Ps = 40.5\%$; $Hes = 0.123$) or regional ($Ps = 49.3\%$; $Hes = 0.164$) distribution (Hamrick and Godt 1997). Other possible causes to explain the high values of genetic variation in North African species could be differences in sample sizes of population sizes respect to their South African congeners, but species form either area of distribution are remarkably similar in terms of these two factors.

It is likely that abiotic factors have also influenced *Androcymbium*'s patterns of isozyme variation. The interplay between patterns of landscape development and changing climates in South Africa since the Cretaceous is reported to have affected current soil and vegetation distributions (Partridge 1997). According to out divergence time estimates, *Androcymbium* began to diversify in South Africa in the Oligocene and the major diver-

sification events for taxa in South Africa occurred between 21.5 mya (nodes 5 in Fig. 2) and 6.2 mya (node 9 in Fig. 2), concurrent with the decrease in the intensity and frequency of rains and the generation of an edaphic mosaic in the mid-late Miocene (Axelrod and Raven 1978) and with the period of largest turnover in plant taxa in Cape Floristic Region (Coetzee et al. 1983). This situation must have allowed the progressive colonization by species adapted to aridity and the inception of different evolutionary lines in the genus. Further diversification in South Africa was probably facilitated by the absence of catastrophic and climatic fluctuations from dry to humid changes in the Plio-Pleistocene (Scott et al. 1997, Cowling et al. 1997). Geographical isolation and absence of gene flow subsequent to these historical contingences may explain the low values of genetic identity between conspecific populations, especially in *A. irroratum*, *A. villosum*, *A. eghimocymbion* and *A. circinatum* (Table 5).

Divergence time estimates based on cpDNA (Caujapé-Castells et al. 2001) and palaeobotanical evidence (Monod 1957, 1971) hint that it is improbable that *Androcymbium* colonized North Africa previous to the late Miocene (some 12 mya, node 'x' in Fig. 2). During this epoch, the progressive replacement of a woodland savanna by an arid landscape in this area (Maley 1980) presumably gave rise to the arid corridor linking North and South Africa (Monod 1971, van Zinderen Bakker et al. 1975), thereby providing the adequate conditions for the spread of xerophytic taxa like *Androcymbium* (Caujapé-Castells et al. 2001). In this context, one feasible explanation of the much higher number of alleles in South Africa than in North Africa is that taxa in South Africa have had much more time to diverge. Very conspicuous inter-specific morphological and isozymic differentiation in South African *Androcymbium* and the existence of aneuploid karyotypic series only in this geographical area (Margelí et al. 1999, Montserrat et al. unpubl. data) favor this 'time' explanation. Concomittantly, different selective pressures in North and South Africa may have also contributed to the contrasting patterns of populational isozyme variation between the two geographical zones. As discussed above, the picture of high inter-specific morphologic, isozymic and cpDNA differentiation, and low levels of genetic variation that emerges for South African *Androcymbium* does suggest that speciation in this area was driven by strong environmental selection and habitat specialization. Subsequent intra-specific perturbations, presumably caused by population bottlenecks and genetic drift, might have added to different degrees to the inter-population differentiation. Quite differently, the uniformity in morphological and isozymic differentiation patterns and the high levels of population genetic variation in North Africa argues for speciation in local isolates (Pedrola-Monfort and Caujapé-Castells 1994, 1996; Caujapé-Castells and Pedrola-Monfort 1997), where differentiation is achieved through stochastic processes mainly. Thus, historical-environmental factors are most important for explaining genetic variation in *Androcymbium* at a large time scale (e.g. the inception of the basal lineages in South Africa or the splitting that gave rise to the south-north disjunction). However, on a shorter geological time scale, the influence of reproductive traits clearly overrides that of historical environmental factors; at this level, our data show that breeding system is the most reliable predictor of the levels of genetic variation.

Given that the geographical distribution of the genus is similar to that of many other plant genera (i.e. *Acacia*, *Capparis*, *Erica*, *Echium-Lobostemon*, *Helichrysum*, *Kochia*, *Olea*), *Androcymbium* provides a reference model to compare and test the relative influence of historical-environmental factors versus biological factors on levels of genetic variation. The application of the obtained patterns to different plant groups might have an outstanding importance for the study and conservation of rare species.

Appendix. Allele frequencies for 17 taxa of *Androcymbium* of South Africa. Taxa abbreviations correspond to the codes in Table 1

Locus/ allele		Taxon																
		ALBA	AUST	BELL	BURC	CAPE	CIRC	CUSP	DREG	EGHI	HANT	HENS	HUNT	IRRO	PULC	POEL	VILL	WALT
Pgi	a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.173	–
	b	–	–	–	–	–	–	–	–	–	–	–	–	0.016	0.081	–	0.009	–
	c	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	d	1.000	–	–	–	–	–	–	–	–	0.162	–	–	–	–	–	–	–
	e	–	–	0.075	–	–	–	–	–	1.000	0.135	–	–	0.837	0.303	–	–	–
	f	–	–	–	–	–	0.453	–	–	–	–	–	–	–	–	–	0.045	–
	g	–	–	0.069	0.981	–	0.047	–	–	–	0.703	–	–	0.146	0.492	–	0.491	0.018
	h	–	–	–	0.019	–	0.405	–	–	–	–	–	–	–	0.015	–	0.036	–
	i	–	–	0.019	–	–	–	–	–	–	–	1.000	–	–	0.008	–	–	–
	j	–	–	0.057	–	–	–	–	–	–	–	–	–	–	–	1.000	–	0.839
	k	–	1.000	0.075	–	–	0.047	–	–	–	–	–	1.000	–	–	–	0.173	–
	l	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–
	m	–	–	0.150	–	–	–	–	–	–	–	–	–	–	–	–	–	0.143
	n	–	–	0.044	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–
	o	–	–	–	–	–	0.047	–	–	–	–	–	–	–	–	–	0.073	–
Aco-1	a	–	–	–	–	1.000	–	1.000	–	–	–	–	–	–	–	–	–	–
	b	0.394	–	–	–	–	–	–	–	–	–	1.000	–	0.402	0.238	–	–	–
	c	–	1.000	–	–	–	–	–	–	–	–	–	–	0.033	–	–	–	–
	d	–	–	–	0.500	–	–	–	–	–	–	–	–	–	–	–	–	–
	e	0.293	–	–	0.500	–	1.000	–	1.000	1.000	1.000	–	1.000	0.565	0.762	–	1.000	–
	f	0.314	–	1.000	–	–	–	–	–	–	–	–	–	–	–	1.000	–	1.000
Aco-2	a	–	–	–	1.000	–	–	1.000	–	–	–	1.000	–	0.288	1.000	–	–	–
	b	1.000	–	–	–	–	–	–	1.000	0.667	–	–	–	0.221	–	–	–	–
	c	–	–	1.000	–	–	1.000	–	–	0.333	1.000	–	1.000	0.490	–	1.000	0.571	1.000
	d	–	1.000	–	–	0.057	–	–	–	–	–	–	–	–	–	–	–	–
	e	–	–	–	–	0.943	–	–	–	–	–	–	–	–	–	–	0.429	–
Aco-3	a	–	–	–	–	–	–	–	–	–	–	–	–	0.143	–	–	–	–
	b	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–
	c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.054	–
	d	–	–	1.000	–	–	–	–	–	–	–	–	–	–	–	1.000	–	0.500
	e	0.468	0.500	–	1.000	0.028	0.952	–	1.000	1.000	1.000	–	0.125	0.842	0.978	–	0.946	0.446
	f	0.532	0.500	–	–	0.972	0.048	–	–	–	–	–	0.875	0.015	0.022	–	–	–

	g	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.054
	h	–	–	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–
Gdh-1	a	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	b	–	–	–	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–
	c	–	–	–	1.000	–	–	–	–	–	–	–	–	–	1.000	–	–	–
	d	1.000	1.000	1.000	–	–	0.920	–	–	0.667	1.000	1.000	–	–	–	1.000	1.000	0.444
	e	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–
	f	–	–	–	–	–	–	–	–	0.333	–	–	–	–	–	–	–	–
	g	–	–	–	–	–	–	–	1.000	–	–	–	–	1.000	–	–	–	0.556
	h	–	–	–	–	–	0.080	–	–	–	–	–	–	–	–	–	–	–
Gdh-2	a	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–	–	–
	b	–	–	–	–	–	–	–	–	–	–	–	–	0.789	–	–	–	–
	c	–	1.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	d	–	–	–	–	–	–	–	–	–	–	–	–	0.211	–	–	–	–
Pgm-1	a	–	–	–	–	–	0.007	–	–	–	–	–	–	–	–	–	–	–
	b	–	–	0.301	–	–	0.420	1.000	–	–	–	–	–	0.676	0.015	–	0.955	–
	c	1.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	d	–	–	–	–	–	0.220	–	–	–	0.824	–	–	0.324	–	–	–	–
	e	–	–	–	–	–	0.067	–	–	–	–	–	–	–	–	–	–	–
	f	–	–	–	–	–	0.007	–	–	–	–	–	–	–	–	–	–	–
	g	–	–	0.006	1.000	1.000	0.280	–	–	–	–	1.000	–	–	0.985	1.000	0.045	0.482
	h	–	–	–	–	–	–	–	–	–	0.176	–	–	–	–	–	–	0.089
	i	–	1.000	0.288	–	–	–	–	1.000	1.000	–	–	0.769	–	–	–	–	0.429
	j	–	–	0.391	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	k	–	–	0.013	–	–	–	–	–	–	–	–	0.231	–	–	–	–	–
Adh	a	–	–	–	–	–	–	–	–	1.000	–	–	–	–	0.154	–	–	0.143
	b	1.000	0.797	1.000	1.000	1.000	1.000	1.000	–	–	1.000	–	1.000	1.000	0.982	1.000	1.000	0.857
	c	–	0.203	–	–	–	–	–	1.000	–	–	1.000	–	–	0.023	–	–	–
Me	a	1.000	–	–	1.000	–	–	–	–	–	0.579	1.000	–	0.241	1.000	–	–	–
	b	–	–	–	–	–	0.096	–	–	–	–	–	–	–	–	–	1.000	–
	c	–	–	–	–	–	0.904	–	–	–	–	–	–	–	–	–	–	0.643
	d	–	–	0.750	–	1.000	–	1.000	–	–	0.421	–	–	0.759	–	–	–	–
	e	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.357
	f	–	–	–	–	–	–	–	1.000	–	–	–	1.000	–	–	–	–	–
	g	–	1.000	0.250	–	–	–	–	–	1.000	–	–	–	–	–	1.000	–	–

Appendix (continued)

Locus/ allele		Taxon																
		ALBA	AUST	BELL	BURC	CAPE	CIRC	CUSP	DREG	EGHI	HANT	HENS	HUNT	IRRO	PULC	POEL	VILL	WALT
Mdh-1	a	–	–	–	–	–	–	–	1.000	–	–	–	1.000	–	–	–	–	0.034
	b	1.000	–	1.000	0.852	1.000	0.904	1.000	–	–	1.000	1.000	–	0.108	0.615	1.000	0.667	0.966
	c	–	1.000	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–
	d	–	–	–	0.148	–	–	–	–	–	–	–	–	0.892	0.385	–	–	–
	e	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.333	–
	f	–	–	–	–	–	0.096	–	–	–	–	–	–	–	–	–	–	–
Mdh-2	a	–	–	–	–	–	–	–	–	–	–	–	0.846	–	–	–	–	–
	b	1.000	–	–	0.963	–	0.725	–	–	–	0.803	1.000	0.154	1.000	0.854	–	0.602	–
	c	–	–	–	–	–	–	0.135	–	–	–	–	–	–	–	1.000	–	–
	d	–	–	–	–	–	–	–	0.630	–	–	–	–	–	–	–	–	–
	e	–	–	–	–	–	–	0.760	–	–	–	–	–	–	–	–	–	–
	f	–	1.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	g	–	–	–	0.037	–	0.275	–	–	–	0.197	–	–	–	0.146	–	0.398	–
	h	–	–	1.000	–	1.000	–	–	–	–	–	–	–	–	–	–	–	1.000
	i	–	–	–	–	–	–	0.105	0.370	1.000	–	–	–	–	–	–	–	–
6pgd-1	a	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–
	b	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–
	c	–	–	–	–	–	–	–	–	–	–	–	–	0.104	–	–	–	–
	d	1.000	1.000	–	–	–	–	–	–	–	–	–	–	0.896	–	–	–	–
	e	–	–	–	–	–	–	–	1.000	–	–	–	1.000	–	–	–	–	–
6pgd-2	a	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	–
	b	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–
	c	–	–	–	–	–	–	–	–	–	–	–	–	0.104	–	–	–	–
	d	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.096
	e	1.000	1.000	–	–	–	–	–	–	–	–	–	–	0.896	–	–	–	–
	f	–	–	0.921	–	–	–	–	–	–	–	–	–	–	–	1.000	–	0.904
	g	–	–	0.079	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	h	–	–	–	–	–	–	–	–	–	–	1.000	1.000	–	–	–	–	–
	i	–	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–
	j	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–

6pgd-3	a	–	–	–	–	–	–	–	–	–	–	–	–	0.160	–	–	–		
	b	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–		
	c	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–		
	d	–	–	–	–	–	–	–	–	–	–	–	–	0.104	–	–	–		
	e	–	–	–	–	–	–	–	–	–	–	–	–	–	0.198	–	–	–	
	f	1.000	1.000	–	–	0.028	0.055	–	–	–	–	–	–	0.896	–	–	0.027	–	
	g	–	–	1.000	–	–	–	–	–	–	–	–	–	–	–	1.000	–	1.000	
	h	–	–	–	0.180	–	–	–	–	–	–	–	–	–	–	–	–	–	
	i	–	–	–	–	0.500	0.096	–	–	–	–	–	–	–	0.434	–	0.107	–	
	j	–	–	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–	
	k	–	–	–	0.620	–	–	–	–	–	–	–	1.000	–	–	–	0.313	–	
	l	–	–	–	0.120	–	0.137	–	–	–	–	–	–	–	–	–	0.116	–	
	m	–	–	–	–	–	0.712	–	–	–	1.000	–	–	–	0.057	–	–	–	
	n	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.438	–	
	o	–	–	–	0.080	0.472	–	–	–	–	–	–	–	–	0.151	–	–	–	
	p	–	–	–	–	–	–	–	1.000	–	–	–	–	–	–	–	–	–	
6pgd-4	a	–	–	–	–	–	–	–	–	0.150	–	–	1.000	–	–	–	–	–	
	b	–	–	–	–	–	–	–	0.778	0.350	–	–	–	–	–	–	–	–	
	c	–	–	–	0.040	–	–	–	–	–	–	–	–	–	–	–	0.295	–	
	d	–	–	–	–	–	–	0.323	–	–	–	–	–	–	–	–	–	–	
	e	–	–	–	–	–	–	–	0.222	0.150	–	–	–	0.109	–	–	–	–	
	f	–	–	–	–	–	–	–	–	–	–	–	–	–	0.066	–	–	–	
	g	0.500	0.508	0.460	–	0.528	1.000	0.677	–	–	–	–	–	0.250	0.566	0.500	0.125	0.981	
	h	–	–	–	–	–	–	–	–	–	–	1.000	–	–	0.066	–	–	–	
	i	–	–	0.540	–	–	–	–	–	–	–	–	–	–	–	0.500	0.455	0.019	
	j	–	0.492	–	–	0.472	–	–	–	–	–	–	–	0.368	0.302	–	–	–	
	k	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.063	–	
	l	0.500	–	–	0.560	–	–	–	–	0.350	0.763	–	–	0.273	–	–	0.063	–	
	m	–	–	–	0.080	–	–	–	–	–	–	–	–	–	–	–	–	–	
	n	–	–	–	0.320	–	–	–	–	–	0.237	–	–	–	–	–	–	–	
6pgd-5	a	1.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

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Addresses of the authors: Núria Membrives, Joan Pedrola-Monfort, Estació Internacional de Biologia Mediterrània – Jardí Botànic Marimurtra, P. O. Box 112, E-17300 Blanes, Girona, Spain. Juli Caujapé-Castells (e-mail: julicaujape@granca. step. es), Jardín Botánico Canario "Viera y Clavijo", Apartado de correos 14 de Tafira Alta, E-35017 Las Palmas de Gran Canaria, Spain.