American Journal of Botany 93(7): 1052–1064. 2006.

THE EVOLUTIONARY HISTORY OF *MELIANTHUS* (MELIANTHACEAE)[1]

H. PETER LINDER,[2,4] TITUS DLAMINI,[3,5] JACK HENNING,[3,6] AND G. ANTHONY VERBOOM[3]

[2]Institute for Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland; and
[3]Department of Botany, University of Cape Town, Rondebosch 7700, South Africa

The evolutionary origins of the morphological and taxonomic diversity of angiosperms is poorly known. We used the genus *Melianthus* to explore the diversification of the southern African flora. *Melianthus* comprises eight species, and a phylogeny based on one nuclear and two plastid genes, as well as a morphological data set, confirmed that the genus is monophyletic. The two earliest diverging lineages are found in relatively mesic habitats, whereas the two terminal clades (an eastern and a western clade), each with three species, favor more arid habitats. The eastern clade is largely restricted to the summer-rainfall parts of southern Africa, and the western clade is found in winter-rainfall region. Molecular dating indicates a mid-Tertiary origin of the genus, with diversification of the eastern and western clades coincident with the Late Miocene–Pliocene uplift of the Escarpment mountains and the establishment of summer aridity along the west coast. The remarkably complex flowers are indicative of sunbird pollination, but many smaller birds can also visit. Speciation may be the consequence of allopatric divergence into edaphic–climatic niches. Divergence in flower and inflorescence morphology might be in response to the divergent pressures for nectar conservation in arid regions coupled with the need for signaling to avian pollinators in generally shrubby vegetation.

Key words: bird pollination; Cape flora; diversification; Melianthaceae; *Melianthus*; molecular dating; South Africa; speciation.

Southern Africa has a rich flora of 20 955 seed plant species (Germishuizen and Meyer, 2003), making up some 8% of the world's flora and over 40% of the African flora (Linder et al., 2005). The region embraces a diversity of environments varying dramatically in terms of topography, altitude, rainfall volume and seasonality, and geology. Overlain on this environmental diversity is a series of distinctive floras, which include the Mediterranean-type flora of the Cape, the Afromontane flora of the eastern Escarpment, the succulent desert flora of the arid west, the steppe grassland of the central plateau, the coastal forest flora of the east coast, and the savanna flora to the north (van Wyk and Smith, 2001).

The origins of the modern day floristic diversity of the region remain poorly understood. Various dramatic vegetation changes have been proposed for the Cenozoic (Axelrod and Raven, 1978), but the underlying evidence is poor. Although it is apparent that the region experienced a series of climatic oscillations during the Quaternary (Scott, 1999; Scott and Vogel, 2000), it is not clear how these affected floristic evolution. One approach to understanding floristic change is through the historical study of the individual taxa making up the flora of interest. For example, there has been extensive research on the evolutionary history of several clades belonging to the Cape flora (Manning and Linder, 1992; Linder and Mann, 1998; Goldblatt et al., 2002; Verboom, Linder, and Stock, 2003; Verboom et al., 2004; Bakker et al., 2005), and the results have been used to develop a scenario of a floristic radiation into a newly emergent winter-rainfall region during the Neogene (Goldblatt and Manning, 2002; Linder and Hardy, 2004; Linder, 2005). In contrast to the extensive work done on the Cape, there have been few attempts to reconstruct the evolution of lineages with broader distributions in southern Africa. Archibald et al. (2005) showed that *Zaluzianskya* arose in the arid parts of southern Africa and subsequently adapted to more mesic conditions in the east, and Mummenhoff et al. (2005) suggested that the seasonal distribution of rainfall influenced differentiation in *Heliophila*.

In this study, we investigate the evolution of *Melianthus*, a genus of eight species belonging to the Melianthaceae. Melianthaceae includes five morphologically quite divergent genera: three in Africa and two in South America. *Melianthus* has a broad distribution in southern Africa, occupying a wide range of habitats and is represented in several centres of floristic endemism (e.g., Gariep, Kamiesberg, Hantam-Roggeveld, Albany, Drakensberg Alpine, Cape Floristic Region [van Wyk and Smith, 2001]). Because six of the eight species have narrow distribution ranges, the genus has the potential to yield valuable insights into the evolution of the southern African flora. Unlike its sister genus, *Bersama*, which is entirely arborescent, *Melianthus* is composed entirely of shrubs (Fig. 1). The genus has several unusual features, including the production of black nectar by some species and highly foetid foliage. The latter has led to widespread ethnobotanical use (Watt and Breyer-Brandwijk, 1962; Kelmanson et al., 2000). There has been extensive phytochemical research on the genus (Anderson, 1968; Anderson and Koekemoer, 1968, 1969; Koekemoer et al., 1970, 1971, 1974; Agarwal and Rastogi, 1976). Several species are also horticulturally interesting (Andrews, 1987).

In this paper, we used morphological and DNA sequence data, sampled from both the nuclear (ITS) and plastid (*trnL-trnF*, *psbA*) genomes, to develop a phylogeny of *Melianthus*,

[1] Manuscript received 5 December 2005; revision accepted 20 April 2006.

The authors thank the directors of B, E, GRA, K, NU, PRE, SRGH, and WIND for loaning their material to the Bolus Herbarium and Cape Nature and the Northern Cape Department of Nature Conservation for permission to collect material in areas under their jurisdiction, and gratefully acknowledge funding from the University of Cape Town and the NRF of South Africa.

[4] Author for correspondence (e-mail: peter.linder@systbot.unizh.ch); phone: +41 44 634 8410; fax: +41 44 634 8403

[5] Current address: Peak Timbers Ltd., Piggs Peak, Swaziland.

[6] Current address: Biology Department, Lehman–CUNY, New York 10468 USA

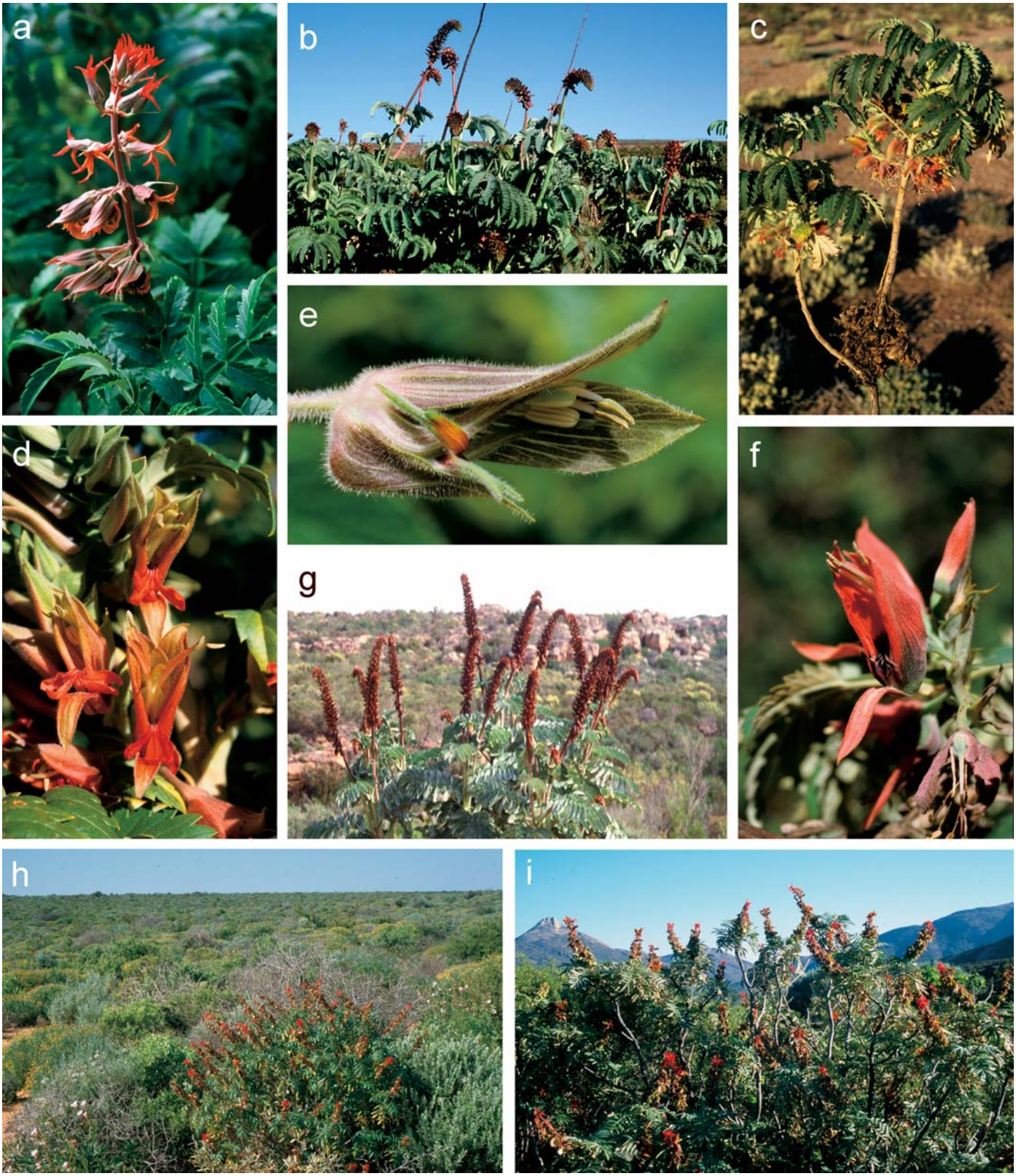

Fig. 1. Morphology and habitats of *Melianthus*. (a) *M. elongatus*, inflorescence with crown of bright red sterile flowers. (b) *M. major*, inflorescences raised on long peduncles. (c) *M. comosus*, inflorescence borne below the leaves. (d) *M. comosus*, flowers with striking red petals and reddish-green sepals. (e) *M. villosus*, flower with green sepals and small orange petals, showing the shallow flowers, with the unilateral nectary in the lower half of the flower. (f) *M. dregeanus*, flowers with red sepals and black center. (g) *M. major* on sandy soils, in the background the sandstone bedrock can be seen. Note massive inflorescences on tall peduncles. (h) *M. elongatus* on the coastal plain on Quaternary sand. (i) *M. pectinatus* in the Kamiesberg, showing the granitic tors in the background. Note in *M. elongatus* and *M. pectinatus* the striking effect of the crown of sterile flowers.

and then used this phylogeny to explore the evolution of the genus. In the context of this phylogeny, we used a molecular clock to infer the time over which the group diversified and to relate the diversification in the genus to paleoclimatic changes in the southern African Neogene. We also explored the possible role of pollinator-specificity in facilitating speciation, but showed that adaptation to different habitats and allopatric isolation constitute more likely explanations for the modern taxonomic and morphological diversity in *Melianthus*.

MATERIALS AND METHODS

DNA sequence data—Twenty accessions representing all species and subspecies of *Melianthus* and four outgroups (*Greyia flanaganii, G. radlkoferi, Bersama lucens, B. swinnyi*) were sampled from plants growing in the field or in cultivation (Appendix). *Bersama* is sister to *Melianthus* (Savolainen et al., 2000), while *Greyia*, though being more distantly related to *Melianthus*, is nonetheless included with the South American genera *Francoa* and *Tetilla* in Melianthaceae (APG, 2003). Voucher specimens of all accessions are housed in the Bolus Herbarium, University of Cape Town (BOL).

Total genomic DNAs were isolated from fresh leaves or from leaves collected into a 2×CTAB solution containing 1% polyvinylpyrrolidone (PVP). Extractions were done according to the protocol of Gawel and Jarrett (1991), with 0.05–0.1 mg PVP powder added during grinding.

Three noncoding DNA loci representing both plastid and nuclear genomes were sampled using PCR. The *trnL-trnF* region (including both the *trnL* intron and the *trnL-trnF* intergenic spacer) and the *psbA-trnH* intergenic spacer of the plastid genome were amplified, respectively, using primers c and f (Taberlet et al., 1991), and primers psbAF and trnHR (Sang et al., 1995). The nuclear internal transcribed spacer (ITS) region was amplified using primers ITS5 and ITS4 (White et al., 1990). Amplification reactions (50 μL) contained 5 mM $MgCl_2$, 1× DNA polymerase reaction buffer (Bioline, London, UK), 0.4 mM total dNTP, 0.2 μM each primer, 0.05 units/μL *Taq* DNA polymerase (Bioline), and 2 μL of template DNA. For ITS, reactions also contained 2% dimethyl sulfoxide (Buckler et al., 1997). Amplifications were done on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, California, USA) with the following thermal profile: 2 min at 94°C; 30 cycles of 1 min at 94°C, 1 min at 52°C (plastid loci) or 55°C (ITS), and 2 min at 72°C; and 7 min at 72°C. PCR products were cleaned using a Qiaquick PCR Purification Kit (Qiagen GmBH, Hilden, Germany) and cycle-sequenced using the ABI PRISM BigDye Terminator Cycle Sequence Kit (Applied Biosystems, Warrington, UK). Sequence reactions were done using the amplification primers and, in some cases, an additional internal primer (for *trnL-trnF* primer e, and for ITS primers ITS3 (White et al., 1990) or 5.8S (Hershkovitz and Lewis, 1996)) was used. Cycle sequencing reactions (10 μL) contained 2.0 μL of BigDye (version 3.0), 0.5× cycle sequence buffer (Applied Biosystems, Warrington, UK), 0.16 μM primer, and 0.5–2.0 μl of PCR product. For ITS, reactions also contained 1% dimethyl sulfoxide. Reactions were run over 25 cycles, each comprising 30 s at 96°C, 15 s at 50°C, and 4 min at 60°C, and the products were run out on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Foster City, California, USA).

Forward and reverse sequences were assembled and edited in SeqMan II version 2.04 (Lasergene Software, DNASTAR, Madison, Wisconsin, USA) before being manually aligned in MegAlign (Lasergene Software). Although alignment required the insertion of several gaps, assessment of sequence homology was largely unambiguous. Four hypervariable regions in the ITS sequences of *Greyia* (corresponding to positions 63–80, 94–127, 169–231, and 409–442 in the ITS sequence of *G. flanaganii*) were exceptional in this regard, proving impossible to align meaningfully to those of Melianthaceae. Rather than discard these sites (which were alignable within Melianthaceae), we coded them as unknown for the *Greyia* outgroups.

For phylogenetic analysis, nucleotide gaps were treated as missing, and the presence/absence of insertions/deletions (indels) coded separately using simple gap coding (Simmons and Ochoterena, 2000) as implemented in the program GapCoder (Young and Healy, 2003). Although most indels so scored were included in subsequent analyses, those flanking long single-base repeats (corresponding to positions 429–430 in the ITS of *M. comosus* 4; positions 184–203 in the *psbA-trnH* of *M. comosus* 4; and positions 657–666 in the *trnL-trnF* of *M. comosus* 4) were excluded due to scoring ambiguity. A 30–31 bp stretch in the *psbA-trnH* sequences (corresponding to positions 102–132 in the *psbA-trnH* sequence of *M. comosus* 4) containing an inversion in some taxa was also excluded from analyses, the presence/absence of the inversion being scored as a single binary character.

The final molecular character matrix, excluding ambiguous gaps and the inversion sequence, comprised a total of 2142 characters (299 informative) consisting of 1430 plastid (133 informative) and 712 (166 informative) nuclear DNA characters.

Morphological data—Morphological characters were scored from 243 *Melianthus* specimens drawn from the following herbaria: B, BOL, E, GRA, K, NU, PRE, SRGH, and WIND (abbreviations follow Holmgren and Holmgren, 1998 onwards). In addition, most taxa were also investigated in the field, in many cases at several populations.

Seed morphology was investigated using field-collected seed as well as seed acquired from Silverhill Seed Company or the seed bank at the National Botanic Gardens, Kirstenbosch, South Africa. Ten seed length and width measurements per collection were measured using a dissecting microscope fitted with an eyepiece graticule. To study seed coat micromorphology, dry seeds were cut into pieces c. 2 × 2 mm, mounted directly on stubs without critical-point drying, then sputter coated with gold and and photographed using a Cambridge S200 scanning electron microscope (SEM) at 10 kV.

Palynological variation was studied using pollen grains acetolyzed according to the method of Erdtman (1952), modified as follows: anthers were excised, soaked overnight in a wetting agent, washed in glacial acetic acid, and digested in an acetolysis mixture (9:1 glacial acetic acid to sulphuric acid). Digestion was achieved by gently heating the mixture in a boiling water bath in a fume cupboard for 10 min. Acetic acid provides a suitable interface between water and acid and stains the pollen grains; consequently, it was used to wash the grains before they were rinsed in distilled water. For light microscopy, acetolyzed pollen grains were mounted in glycerine jelly and fixed semipermanently in paraffin wax. They were observed using a Zeiss Standard 25 light microscope and photographed on a Zeiss Axioskop microscope using bright field optics. For SEM, acetolyzed grains were mounted on stubs, dried by allowing the 70% alcohol they were suspended in to evaporate, and sputter coated with gold, before being photographed.

The final morphological character matrix comprised 54 characters, all parsimony informative (Appendices S1 and S2, see Supplemental Data accompanying the online version of this article). The data matrix and resulting trees have been submitted to TreeBase (SN 2741) at http://www.treebase.org/treebase/.

Phylogenetic analyses—The plastid (*trnL-trnF* + *psbA-trnH*) DNA, nuclear (ITS) DNA and morphological partitions were analyzed using parsimony, both separately and in combination. Two combined analyses were done, one using only the two molecular partitions and a second based on all three data partitions. For the latter analysis, the molecular data sets were pared down to include a single representative of each *Melianthus* species, as well as a single species each of *Bersama* and *Greyia*. All searches were conducted using the Branch and Bound search algorithm implemented in PAUP* version 4.0b10 (Swofford, 2002). Characters were treated as unordered and equally weighted (Fitch, 1971). Character support for nodes was evaluated using 1000 bootstrap replicates (Felsenstein, 1985). Bootstrap searches were conducted using a heuristic search procedure with a simple addition sequence and with MAXTREES set to 1000.

Molecular dating—We tested whether the molecular evolution in *Melianthus* was clocklike by evaluating the likelihood of the total evidence topology, with and without a clock assumption, in the context of the combined three-gene data and a model determined as appropriate using Modeltest 3.6 (Posada and Crandall, 1998). The resulting likelihoods were compared using a log-likelihood ratio test.

In the absence of a clock, the ages of speciation events were estimated using a relaxed Bayesian clock as implemented in Multidivtime (Thorne and Kishino, 2002) and following the protocols suggested by Rutschmann (2004). We selected the F84 model of molecular change. This model was parameterized by the baseml module of the program PAML (Yang, 1997). The branch lengths for the chosen cladogram plus our data matrix, as well as a variance–covariance matrix, were estimated using Estbranches (distributed with Multidivtime). The tree was calibrated using the ages calculated by Wikström et al. (2001) for the node linking *Bersama* with *Greyia*. Wikström et al. obtained a maximum age of 67 million years (My) for this node using ACCTRAN, and a minimum age of 59 My using ML, with a standard deviation of 7 My. We set these dates as upper and lower bounds, but did not take the standard deviations into account.

These dates have an unknown error in them, and should be regarded as exceedingly rough estimates. Using as additional priors the number of time units between the tip and the root of the tree (rttm), the distribution of the rate at the root node, and a constant for the Brownian motion, we used Multidivtime to estimate the posterior distribution of the node ages. We ran the Markov Chain for 10^6 generations, with every 10th generation being sampled, and the first 10 000 samples being discarded. Rttm, the a priori expected number of time units between tip and root, was set to 1.5, and the mean of the prior distribution of the rate at the root node was set to 0.09. The latter was calculated by Estbranches as the mean amount of evolution from the root node to the tips, divided by the arbitrarily set rttm. Brownmean, the prior for the brownian motion constant, was set to equal the product of Brownmean and rttm, and to lie between 1 and 2 as suggested by Rutschmann (2004). Due to the relatively small size of the data set, the data were not partitioned but analyzed under a single model. The analysis was run twice and the results compared to establish whether convergence had occurred.

The hypothesis that there might have been a slowdown in the speciation rate was tested with the program Gammastatistic (Griebeler, 2004).

Ecological reconstructions—Species distributions were mapped using herbarium specimen locality data, as well as field records made over several field seasons. Using these distributions, annual rainfall ranges for each species were abstracted from the rainfall models of the South African Weather Bureau (Schulze, 1997), the ranges being coded categorically in steps of 200 mm, from 0 to 1200 mm. Rainfall seasonality was coded simply as "summer-dry" and "winter-dry." The bedrock associations of each species were coded according to the major bedrock types (e.g., granite, recent sand, shale, basalt, cave sandstone, or Table Mountain sandstone) according to the geological maps of southern Africa. Bedrock scoring was based on field observation, herbarium specimen label data, and inferences drawn on the basis of species' distribution ranges. To accommodate ancestral polymorphism, the data were coded categorically as presence-only (Hardy and Linder, 2005). Both DELTRAN and ACCTRAN optimization, as implemented in MacClade 4.0, were used. Although this approach does not provide an estimate of the level of confidence in the resulting optimizations, it does cover the full range of equally parsimonious solutions.

Pollinator observations—Pollinator specificity in *Melianthus* was evaluated on the basis of observations made in the field during August–December 2002. During this period, all species except *M. insignis* and *M. villosus* were seen flowering in the field, and the full set of bird species visiting each investigated population of *Melianthus* was noted. Based on our observations, it was not possible to distinguish among bird species that are effective pollinators and those that are not. Using accounts in Maclean (1993), bird visitors were subsequently categorized as being (1) primarily nectarivorous, (2) occasionally nectarivorous, or (3) non-nectarivorous.

The nectar of all species except *M. insignis* was sampled, the sample for each species typically being drawn from 10 individuals (one flower per individual) belonging to a single population. The only exception was *M. villosus* for which the 10 replicates came from a single multibranched specimen in cultivation. Nectar was extracted with a 10–100 μL Finnpipette micropipette (Labsystems, Helsinki, Finland), this also being used to estimate volumes. Sucrose readings were calculated using a hand-held refractometer (Atago, 32–10 Honcho, Itubashi-ku, Japan), which measures nectar concentration in terms of equivalent percentage sucrose ratios.

RESULTS

Phylogeny—Consensus topologies obtained from the various parsimony analyses are shown in Fig. 2 with associated tree statistics listed in Table 1. The levels of homoplasy are remarkably low.

All analyses provide strong support for the monophyly of *Melianthus*. Although the trees based on separate analyses of the three data partitions show some incongruence within *Melianthus* (Fig. 2a–c), this conflict is in no case strongly supported. Plastid support for the monophyly of *M. dregeanus* + *M. elongatus* + *M. insignis* is strongly contradicted by the nuclear and morphological topologies (96% and 91% bootstrap, respectively), but is itself weak (60%) and dependent on a single substitution character (position 1445 in *trnL-trnF*). Similarly, although the morphological data contradict strong plastid support (97% and 95%) for the early divergence of *M. major* and *M. villosus* in *Melianthus*, support for this disagreement is weak (55% and 57%). Finally, where the nuclear data provide strong support (91%) for a sister relationship between *M. elongatus* and *M. gariepinus*, morphology provides weak support (62%) for a contradictory sister relationship between *M. pectinatus* and *M. gariepinus*. When only nodes with bootstrap support >62% are considered, all conflict disappears, suggesting a general lack of supported or "hard" conflict.

Within *Melianthus*, identical relationships are resolved by the combined molecular and total evidence data sets (Fig. 2d). Largely on the strength of the plastid data, *M. major* (Fig. 1b, g) is identified as sister to the rest of *Melianthus*, within which *M. villosus* is resolved as sister to a clade comprising two clades, one comprising three species from the western part of southern Africa (western clade) and the other comprising three species centered on the eastern and central plateau, although *M. comosus* reaches the coastal mountains both in the southern Cape and along the western escarpment (eastern clade). Support levels are generally strong, except for the grouping of the western and eastern clades.

Molecular dating—The molecular clock was rejected. Replicate dating runs using a relaxed Bayesian clock produced very similar results, suggesting that both runs reached stationarity. The result of one of these analyses is shown in Fig. 3 with the results summarized in Table 2. Partly due to our reliance on a single calibration point, the variances surrounding our age estimates are large. In general, the data indicate an Oligocene divergence between *Melianthus* and *Bersama*, followed by an early Miocene differentiation of *M. major* and *M. villosus*. The eastern and western clades diverged around 15 Mya. Within these two clades, the bulk of speciation happened prior to 8 Mya, though there is no significant slowdown in the diversification rate.

Habitat reconstruction—The annual rainfall ranges scored for the extant species indicate that habitats vary from arid (<300 mm) to mesic (>800 mm). ACCTRAN and DELTRAN optimizations of these data lead to contrasting interpretations (Fig. 4). ACCTRAN identifies an intermediate rainfall habitat (600–800 mm) as ancestral in the genus, with adaptation to drier conditions (<600 mm) evolving subsequently (Fig. 4: node 6) and becoming especially pronounced in the western clade. Adaptation to wetter conditions also occurs twice, in *M. villosus* and *M. insignis*. By contrast, DELTRAN identifies a wet habitat (800-1200 mm) as being ancestral in the genus, with two transitions to drier habitats, in *M. comosus* and in the *M. pectinatus* lineage (Fig. 4, node 2).

For rainfall distribution and bedrock type, the DELTRAN and ACCTRAN optimization are identical. Both identify a summer (winter-dry) rainfall regime as ancestral in the genus (Fig. 4, node 7) with a shift in rainfall seasonality at the base of the western clade (Fig. 4, node 2). Today, three species are restricted to the winter-rainfall zone, four to the summer rainfall zone, and only *M. major* is found in both. The ancestral bedrock type is ambiguous, with the common ancestor of the eastern and western clades occurring on granitic bedrock.

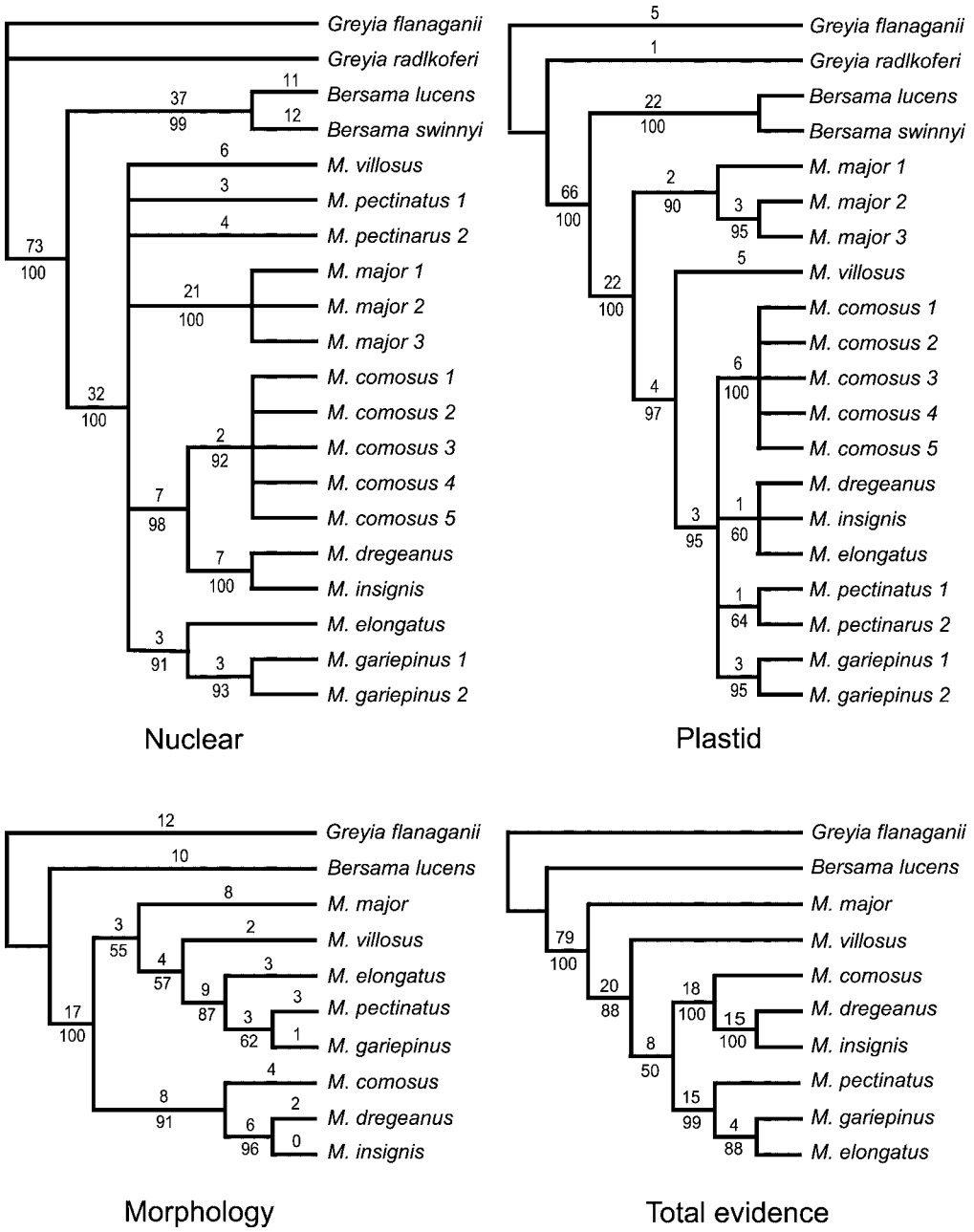

Fig. 2. Consensus trees of the phylogenetic analyses of *Melianthus*. (a) Nuclear phylogeny (strict consensus), (b) plastid phylogeny, (c) morphological phylogeny, (d) total evidence tree. The values above the branches indicate the number of character changes, the values below are bootstrap results.

TABLE 1. Tree statistics for three separate (plastid DNA, nuclear DNA, and morphology) and two combined (molecular, and molecular plus morphology) analyses of *Melianthus*. *N* = number of fundamental trees found, CI = consistency index, RI = retention index.

Character set	*N*	Length	CI	RI
Plastid (*trnL-trnF* + *psbA-trnH*)	1	166	0.98	0.98
Nuclear (ITS)	25	232	0.91	0.93
Molecular (plastid + nuclear)	1	400	0.93	0.95
Morphology	1	95	0.72	0.70
Combined (molecular + morphology)	1	462	0.90	0.74

Pollination and nectar volumes—Field observations suggests that most species of *Melianthus* are visited by a broad range of bird species, and many avian visitors, especially nectarivores and occasional nectarivores, visit several *Melianthus* species (Table 3). This is certainly true for the most ubiquitous visitors to *Melianthus*, such as the lesser double-collared sunbird *Cinnyris chalybeus* and Cape whiteye *Zosterops pallidus*. For example, at a site near Montagu in the western Cape, where *M. major* and *M. comosus* co-occur, observations made over a period of 33 h suggest that the *Z. pallidus* accounts for about 50% of visits to flowers of both species.

Most variation in the visits of primary nectarivores to different species of *Melianthus* can be explained by a lack of

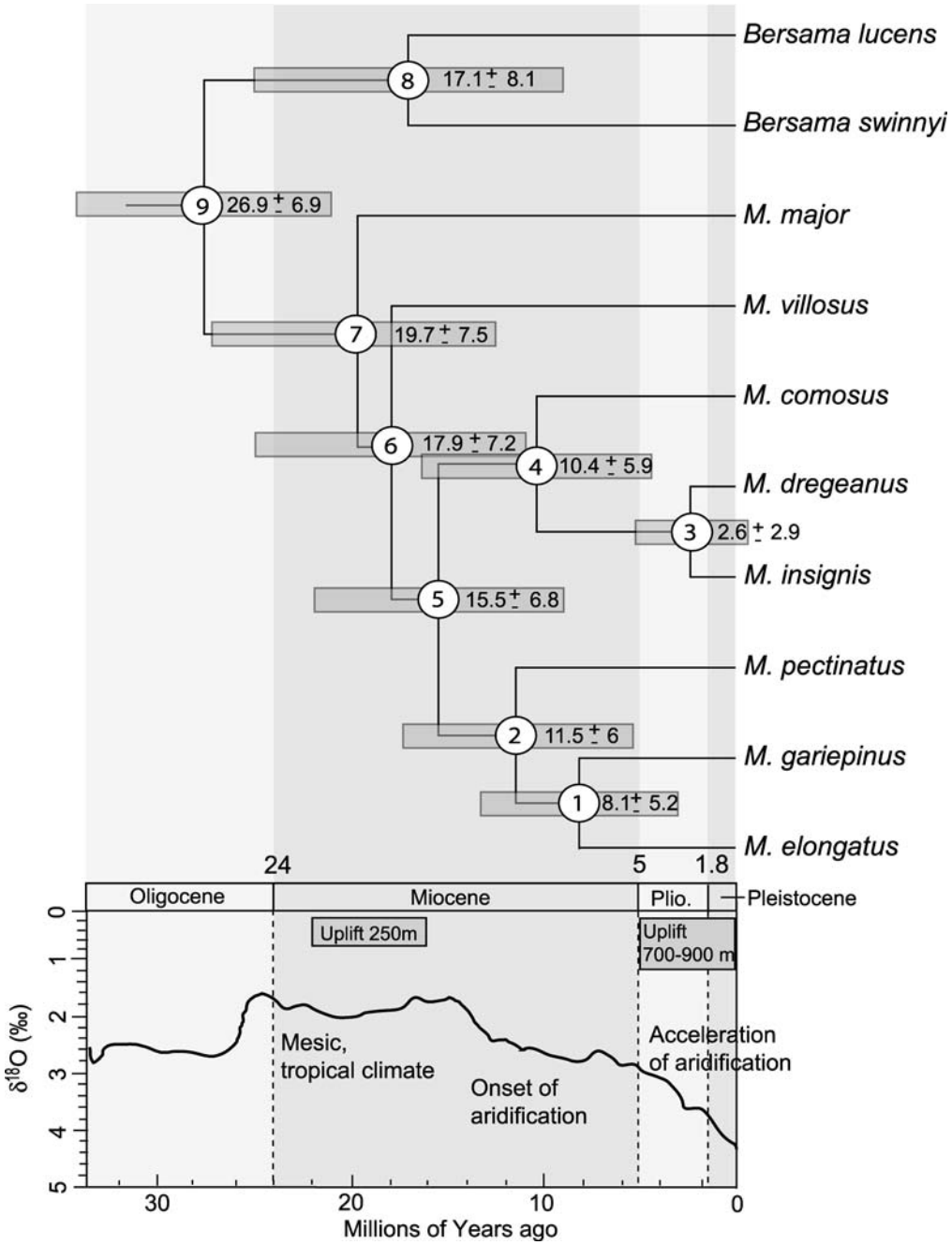

Fig. 3. Phylogram of *Melianthus* with estimated node ages (±SD indicated by the shaded bars) in millions of years. The gross environmental changes are mapped below the phylogram, tracking the evolution of the genus through time. Node numbers are ringed.

sympatry between the plant and bird species. For example, four species of *Melianthus* are not visited by the greater double-collared sunbird *Cinnyris afer* because they occur outside the natural range of the bird. Overall, 63% of nonvisits to *Melianthus* by primary nectarivores are accounted for by failure of plant and pollinator to co-occur geographically. This explanation works slightly less well for occasional nectarivores (39%) and poorly for non-nectarivores (11%).

Nectar volumes varied considerably among the species (Table 4), being greatest in *M. major* (81.0 μL), *M. villosus*, and *M. dregeanus* and lowest in *M. gariepinus* (14.6 μL). Sucrose concentration varied less than nectar volume, ranging between 9.7% and 13.5%.

DISCUSSION

Phylogeny—Our data strongly support the monophyly of *Melianthus* and provide a well-resolved and strongly supported phylogenetic hypothesis of species relationships within the genus. Although there is some conflict among the data

TABLE 2. Age estimates of the interior nodes of the phylogeny of *Melianthus*, as obtained from Multidivtime. Nodes are numbered as in Fig. 3.

Node	Mean age (Mya)	Standard deviation (My)	95% Confidence range (Mya)
1	8.12	5.19	1.26–20.77
2	11.51	5.98	2.69–25.22
3	2.55	2.87	0.05–10.60
4	10.40	5.89	2.05–24.13
5	15.47	6.83	4.41–30.26
6	17.25	7.18	5.28–32.47
7	19.67	7.54	6.66–35.3
8	17.10	8.15	3.61–34.01
9	26.90	6.88	14.25–40.59

partitions on which our analyses are based, this conflict is consistently weak, suggesting that it is the combined result of limited character sampling and stochastic sequence variation (Mason-Gamer and Kellogg, 1997; Eldenäs and Linder, 2000).

The magnitude of support for the monophyly of *Melianthus* is unsurprising: the genus is very different from its relatives, *Bersama* and *Greyia*, and has previously even been separated at family level (Doweld, 2001). The latter two genera are tropical trees, with woody trunks. Although *Greyia* has, like *Melianthus*, showy, red, bird-pollinated flowers, these are actinomorphic (Dahlgren and van Wyk, 1988; Ronse Decraene and Smets, 1999). *Bersama* has zygomorphic flowers, with nectaries that are, as in *Melianthus*, sometimes unilateral (Verdcourt, 1950). However, the flowers are relatively small. Within *Melianthus*, the identification of *M. major* and *M. villosus* as early-diverging elements suggests that the large, long-stalked, terminal inflorescences (Fig. 1b, c), that are held high above the large leaves, represent the plesiomorphic form in the genus. Also, the presence of highly distinctive, single stipules in *M. major* as well as *Bersama* suggests that these, rather than the more common paired stipules represent the primitive state. Solitary stipules are unusual in angiosperms as a whole (Roth, 1949).

The remainder of the genus is divided into two clades. The western clade, with three species restricted to the western margin of southern Africa, is distinguished by having the inflorescences capped by a colorful crown of sterile flowers (Fig. 1a, h, i), and by acutely lobed fruit. In this lineage, as in *M. major* and *M. villosus*, the inflorescences are erect and borne above the foliage; however, their stalks are not as long stalked and they are therefore less prominent. In contrast, the eastern clade, with three species from the central and eastern part of southern Africa, is diagnosed by having "nodding," axillary inflorescences, that are borne beneath the apical leaves (Fig. 1c). Furthermore, the sepals have large distinctive red to black blotches near the base (Fig. 1f).

Timescale of diversification—Molecular dating is subject to error from a diversity of sources (Bell and Donoghue, 2005), some of which may affect our efforts to date divergence times in *Melianthus*. First, accurate dating requires that the underlying phylogenetic hypothesis is correct and that this phylogeny is tracked by all the genes sampled. This is probably true for our study because our phylogeny is robust and the limited incongruence amongst our DNA data partitions is weakly supported and most likely reflects stochasticity in sequence variation. Second, under rate correction, nodal age

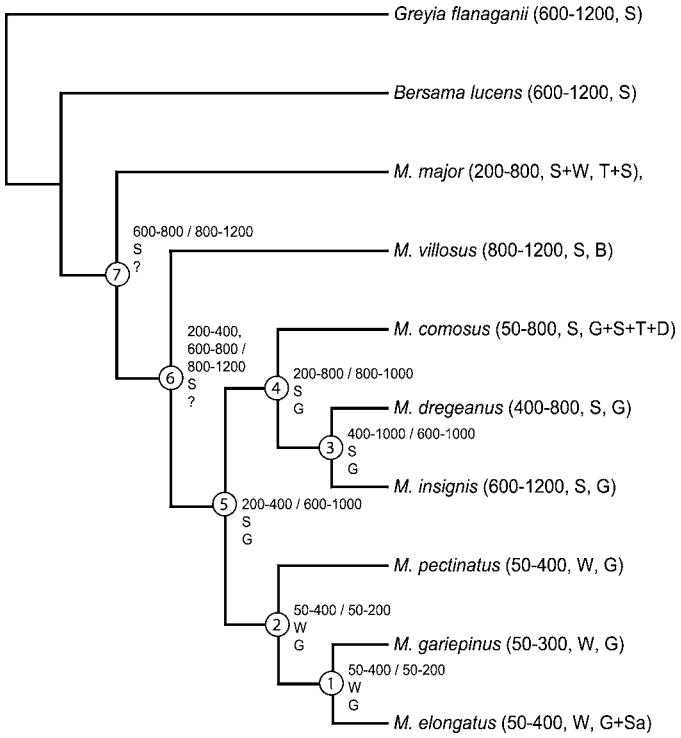

Fig. 4. Phylogeny of *Melianthus* with ecological attributes mapped. *Attribute abbreviations*: rainfall volume in mm per year (ACCTRAN/DELTRAN), rainfall distribution (S = Summer or W = Winter), bedrock (B = Basalt, D = Dolorite, G = Granite, S = Shale, Sa = Sand, T = Table Mountain Sandstone, ? = ambiguous optimization).

estimation may be influenced by incomplete species sampling (Linder et al., 2005). This may be problematic given our scant sampling of *Bersama* and *Greyia*. However, this problem may be largely offset by our use of a relaxed Bayesian clock, which has been found to introduce only minor distortions compared with some other methods (Linder et al., 2005). Third, use of an under parameterized model of sequence evolution could systematically distort branch lengths from the tips to the root node (Sanderson, 1990). Although we do not offer a comprehensive solution to this problem, we have minimized the risk of such distortion by selecting the F84 model, the most complex implemented in Multidivtime. The final source of error is calibration error (Conti et al., 2004; Heads, 2005). In our study, this error is a potential problem because we lack fossil references and so rely exclusively on secondary calibration points. The use of secondary calibrations can result in compounded error, with the result that variances on age estimates are greatly increased (Graur and Martin, 2004; Hedges and Kumar, 2004). The ages estimated by Wikström et al. (2001) are derived from a single calibration point and may contain substantial error, the magnitude of which is unknown. Although we have used both their minimum and maximum age estimates for our calibration node, we have not take into account any error on these ages. Consequently, the substantial variances on our age estimates are almost certainly narrower than they should be (Table 2).

Notwithstanding the limitations involved, our data provide a broad, if somewhat tentative, timescale for the diversification of *Melianthus* (Fig. 3). The differentiation of *Melianthus* from

TABLE 3. Observations of bird visitors to *Melianthus* species. Although no pollinators were actually observed feeding at *M. gariepinus*, dusky sunbirds were in the vicinity and flowers showed signs of recent visitation: thus visitation by dusky sunbirds is tentatively inferred. + = bird seen visiting; O = bird not seen; **O** = bird and *Melianthus* species sympatric; $\underline{\text{O}}$ = bird and *Melianthus* species parapatric (ranges touching); *O* = bird and *Melianthus* species allopatric.

Pollinator species	*Melianthus* species pollinated						No. species visited by pollinator	No. species not visited by pollinator		
	major	*elongatus*	*pectinatus*	*gariepinus**	*comosus*	*dregeanus*		Sympatric	Parapatric	Allopatric
Nectarivores										
Lesser double collared sunbird (*Cinnyris chalybeus*)	+	+	+	$\underline{\text{O}}$	+	+	5	0	1	0
Greater double collared sunbird (*Cinnyris afer*)	+	*O*	*O*	$\overline{O}$	$\underline{\text{O}}$	+	2	0	1	3
Amethyst sunbird (*Chalcomitra amethystina*)	+	*O*	*O*	*O*	$\underline{\overline{\text{O}}}$	**O**	1	0	1	3
Orange-breasted Sunbird (*Anthobaphes violacea*)	**O**	*O*	*O*	*O*	+	*O*	1	1	0	3
Dusky sunbird (*Cinnyris fuscus*)	+	+	+	+	+	*O*	5	0	0	1
Malachite sunbird (*Nectarinia famosa*)	+	**O**	+	*O*	**O**	+	3	2	0	1
Occasional nectarivores										
Cape white eye (*Zosterops pallidus*)	+	+	+	$\underline{\text{O}}$	+	+	5	0	1	0
Cape rock thrush (*Monticola rupestris*)	**O**	*O*	*O*	$\overline{O}$	+	**O**	1	2	0	3
Southern masked weaver (*Ploceus velatus*)	+	**O**	**O**	$\underline{\text{O}}$	+	**O**	2	3	1	0
Cape weaver (*Ploceus capensis*)	+	**O**	**O**	$\overline{O}$	+	+	3	2	0	1
Speckled mousebird (*Colius striatus*)	**O**	**O**	+	*O*	+	**O**	2	3	0	1
Red faced mousebird (*Colius indicus*)	**O**	**O**	$\underline{\text{O}}$	*O*	+	**O**	1	3	1	1
Red winged starling (*Onychognathus morio*)	+	+	+	*O*	+	**O**	4	1	0	1
Pied starling (*Sturnus bicolour*)	**O**	+	$\underline{\text{O}}$	*O*	+	**O**	2	2	1	1
Cape bulbul (*Pycnonotus capensis*)	+	+	+	*O*	+	*O*	4	0	0	2
Black-eyed bulbul (*Pycnonotus capensis*)	+	*O*	*O*	*O*	+	+	3	0	0	3
Non-nectarivores										
Cape robin (*Cossypha caffra*)	**O**	**O**	**O**	$\underline{\text{O}}$	+	**O**	1	4	1	0
Pale winged starling (*Onychognathus nabouroup*)	$\underline{\text{O}}$	+	+	$\overline{\text{O}}$	+	*O*	3	1	1	1
Cape sparrow (*Passer melanurus*)	$\overline{\text{O}}$	**O**	**O**	**O**	+	**O**	1	5	0	0
Cape canary (*Serinus flaviventris*)	**O**	**O**	$\underline{\text{O}}$	*O*	+	**O**	1	3	1	1

Bersama dates from the Oligocene, when climates may have been similar to the modern, with extensive glaciation of Antarctica (Zachos et al., 2001). Within *Melianthus*, the differentiation of *M. major* and *M. villosus* followed in the early Miocene, a wetter and warmer period than the Oligocene, and one in which the eastern Escarpment was uplifted by about 250 m (Partridge and Maud, 2000). The emergence of the western and eastern clades is dated to around 15 My, almost contemporaneous with the initiation of further glaciation on Antarctica (Zachos et al., 2001), which subsequently led to a drop in sea surface temperatures, strengthening of the south Atlantic high pressure cell, and consequent establishment of a distinct winter rainfall regime along the southern African west coast (Linder, 2003). A continued drop in sea surface temperatures (Zachos et al., 2001) through the late Miocene most likely accentuated the east–west climatic gradient. Within *Melianthus*, most speciation was completed by 8 My, though *M. dregeanus* and *M. insignis* diverged later, possibly in association with a second, more substantial phase of uplift (700–900 m) during the Pliocene (Partridge and Maud, 2000).

TABLE 4. Nectar volume and sucrose concentration (mean ± SE) in *Melianthus* flowers. All values are based 10 measurements, typically from 10 flowers on different plants.

Species	Nectar volume (μL)	Concentration (% sucrose)
M. major	81.0 ± 7.2	10.2 ± 0.2
M. villosus	60.0 ± 4.3	11.6 ± 0.1
M. dregeanus	60.3 ± 3.7	11.5 ± 0.4
M. comosus	41.7 ± 5.1	9.7 ± 0.2
M. pectinatus	45.0 ± 4.1	13.5 ± 0.2
M. elongatus	41.9 ± 2.9	13.1 ± 0.4
M. gariepinus	14.6 ± 1.8	12.7 ± 0.5

Ecological background—The appearance of *Melianthus* may be tied to the cool, dry conditions of the Oligocene. Although there are few records of the climate and continental vegetation of this period, and none from southern Africa (Scott et al., 1997), there are some indications of seasonal aridity (Leopold et al., 1992). These conditions may have provided a selective advantage to perennial herbs occupying areas with groundwater, like stream-margins and damp hollows. In this context, the ability to die back to a persistent rootstock, a trait found in both *M. major* and *M. villosus*, would be advantageous, allowing plants to resprout when conditions were favorable. This may have been a more robust strategy than the plesiomorphic treelike habit found in *Bersama* and *Greyia*.

Our reconstructions of ancestral rainfall habitats imply contrasting patterns of arid adaptation in *Melianthus*. ACCTRAN suggests that the genus adapted to aridity early in its history, with later switches to moist environments, whereas DELTRAN projects a wetter habitat for the ancestral lineage, with adaptation to aridity occurring twice subsequently. However, the observed rainfall range for *M. major* probably misrepresents the water requirement of this species. Whereas *M. major* may be found in areas of quite low rainfall, it is restricted to streamlines and ditches where it has access to ground water. Consequently, it appears to require more moisture than is indicated by its rainfall score. If the rainfall range of *M. major* is rescored to reflect its high moisture

requirement, then ACCTRAN and DELTRAN produce similar, high rainfall optimizations for the basal nodes. Thus, we infer that the genus arose in a high rainfall (or at least a damp) environment (600-1200 mm) and that the switch to aridity occurred three times: in *M. major*, in *M. comosus*, and in the ancestor of the western clade. Whereas the latter two lineages may be considered arid-adapted, the first is able to persist in arid conditions only through its exploitation of ground water.

The onset of warmer, moister conditions in the early Miocene, paired with uplift along the eastern Escarpment, appears to have provided the opportunity for *M. villosus* to evolve. The appearance of a new montane habitat, trapping moisture off the Indian Ocean allowed this species to escape the ground water niche occupied, for example, by *M. major*. Today, *M. villosus* is restricted to the mid-altitude belt of the Kwazulu-Natal-Lesotho Drakensberg, where it receives the highest rainfall volumes of any *Melianthus* species.

Rainfall seasonality varies markedly across southern Africa. Most of the region is characterized by wet summers and dry winters, but the western seaboard and the south coast receive their rain in winter (Schulze, 1997). The transition between these rainfall zones is sharp and underlies a correspondingly pronounced floristic boundary (Linder, 2003). Our reconstructions indicate that *Melianthus* arose in the summer rainfall zone and has persisted there, with four modern species being restricted to summer rainfall environments: *M. villosus* and the three eastern clade species. Within the eastern clade, differentiation of the arid-adapted *M. comosus* was probably powered by continental uplift during the Miocene. This would have blocked the passage of orographic rain to the central interior, generating a dry, summer rainfall environment. This is currently exploited by the widespread *M. comosus*, whereas the related *M. dregeanus* and *M. insignis* occupy moister environments near the Escarpment edge.

The divergence of the western clade from the eastern clade marks the occupation of summer-arid environments by *Melianthus*. The occupation of such environments is clearly derived in the genus and coincides temporally (ca. 15 My) with events leading to the establishment of a winter rainfall regime along the southern African west coast. Such a nested pattern, in which species restricted to arid, summer-dry conditions are nested within a clade characterized by wetter summer- or all-year rainfall conditions has already been documented for a number of taxa (Linder and Mann, 1998; Verboom et al., 2003; Bakker et al., 2005). Although various lineages, most notably the ruschioid Aizoaceae (Klak et al., 2004) have radiated spectacularly in the winter rainfall zone, *Melianthus* has yielded just three species there.

The use of bedrock to characterize the substrate in which plants grow is questionable because it is often a poor indicator of soil-type. However, optimization of bedrock type indicates a preference for granite, despite this not being the most common bedrock in southern Africa. The only species restricted to other bedrock types are *M. villosus*, which occurs on basaltic soils, and *M. major*, which is found on Table Mountain sandstone and shale (Fig. 1g).

Pollination—*Melianthus* has a typical avian pollination syndrome (Proctor et al., 1996): vivid, red floral colors, large flowers with a hardened corona featuring stiff filaments (Fig. 1d–f), and abundant nectar of low sucrose concentration (9.7–13.5%). As nectarivorous African birds are incapable of hovering, the robust inflorescence stalks (Fig. 1a–c) are also typical. Most likely, the black color of the nectar of *M. comosus* and *M. elongatus* contributes to polychromatism in the flowers, forming the tricolor "parrot coloration" of Faegri and van der Pijl (1979).

We suggest that the pollination system of *Melianthus* is of the generalist ornithophilous type. Although sunbirds (Nectariniidae) are generally cited as the principal pollinators of *Melianthus* (Scott-Elliot, 1890; Von Marilaun, 1895; Burtt Davey, 1932; Vogel, 1954), many other avian visitors have been reported (Marloth, 1908, 1925; Skead, 1967; Maclean, 1993). Our field observations confirm these reports in showing that most species are visited by a broad array of bird species, including high proportions of the nectarivore species with which they are sympatric. Because our observational data are of an anecdotal nature, we cannot distinguish which bird visitors are effective in pollination and which are not. The shallow, open form of the flowers (Fig. 1d–f) suggests, however, that most bird species should be able to access the nectar, while the protrusion of the sexual parts and the versatile pedicels (which enable the flowers to tip downward) suggests that pollen should be effectively transferred to and from the heads of most birds that enter the flowers. This is especially true for shorter-billed birds such as the Cape whiteye, *Zosterops pallidus*, which is a particularly frequent visitor to most species of *Melianthus*, regardless of floral form. The observation that most *Melianthus* species are visited by a majority of sympatric nectarivores suggests, then, that the flowers do not filter bird visitors and instead employ a generalist bird pollination syndrome. We note that there are some instances where potential bird pollinators were not observed to visit particular *Melianthus* species, despite an overlap in distribution ranges. However, these are readily attributable to the limited scale of our sampling: our bird visitor data are based on observations at a limited number of populations of each *Melianthus* species, over just a single flowering season.

A generalist pollination syndrome suggests a limited role for pollinator selection in driving the floral evolution and speciation (Waser, 1998). Pollinator specialization has been demonstrated in many southern African families and genera (Johnson and Steiner, 2000, 2003), commonly resulting in tight linkages between pollinator shifts, evolution of floral morphology, and speciation (Johnson, 1996). A lack of evidence for pollinator specialization in *Melianthus*, then, poses two questions: what drives the diversification in floral and inflorescence structure in the genus, and what drives speciation?

Floral evolution—Although *Melianthus* has remarkably complex flowers, evidence for a generalist avian pollination system suggests that the substantial variation in floral and inflorescence structure may not be related to the attraction of specialist pollinators. Alternative explanations include the protection of nectar against desiccation and a general need for plants to attract avian pollinators.

Our data suggest that interspecific variation in nectar volume is related to the aridity of the habitats in which the different species occur (Fig. 5). We recorded the largest nectar volumes in *M. major*, which grows in wet hollows and seepages with perennial or near-perennial groundwater, and the smallest volumes in *M. gariepinus* from the arid southern Namib Desert. Our estimates of nectar production are, however, based on measurements from single populations and so fail to account

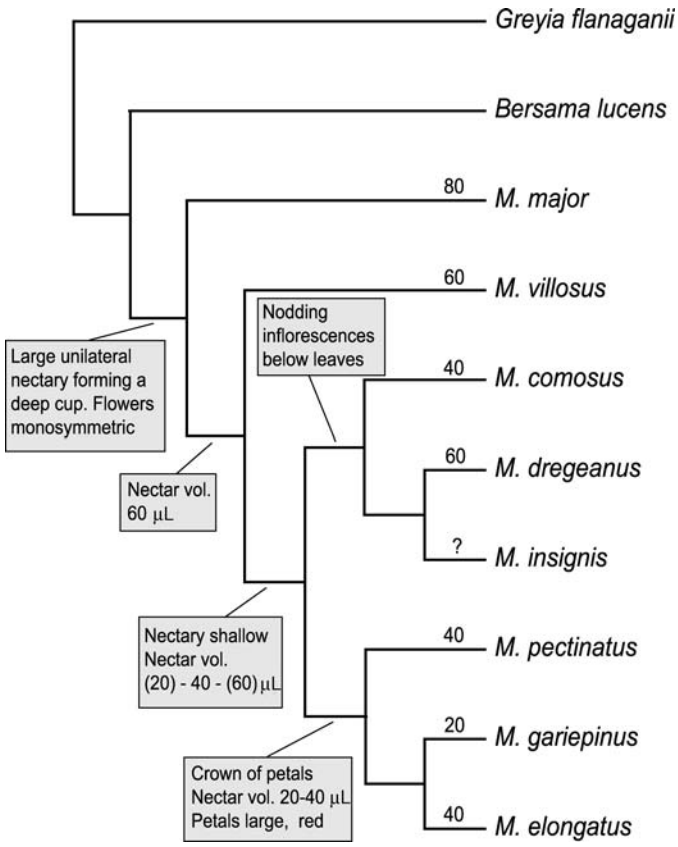

Fig. 5. Phylogeny of *Melianthus* with inflorescence attributes mapped. The nectar volumes recorded for each species are listed on the terminal branches, the attribute changes marked where they occur.

for interpopulational variation in nectar production. Additionally, because our nectar readings were taken from unbagged flowers, it is possible that the observed differences are due to differential levels of nectar removal by bird visitors rather than intrinsic variation in nectar production among species. If the interspecific variation in nectar volume were the result of erratic nectar removal by birds, however, we would expect the species-specific standard errors to be broader than they are, especially because our replicate measurements were generally taken from flowers on different plants.

The nectaries in *Melianthus* are unusual in being found only on one side of the ovary (Fig. 1e), where they are embedded in a prominent mentum (Ronse Decraene et al., 2001). Although consistently cuplike, they vary from being deep, with a high rim, to quite shallow. The shape of the nectary cup is related to the volume of nectar produced, with *M. major* and *M. villosus* having the deepest nectary cups. If nectar production is limited by water availability, changes in nectary shape and size may reflect adaptation to increasingly arid conditions. The shift of the inflorescence from a terminal position to below the leaves (Fig. 1c), as seen in the eastern clade, may be related to the protection of nectar against desiccation.

In the western clade, the evolution of an unusual crown of sterile flowers (Fig. 1a) may serve to enhance pollinator attraction. These plants often occur scattered in the shrubby vegetation and, at least to the human eye, the crown of red, sterile petals greatly enhances their visibility (Fig. 1h, i). This may be necessary given the low nectar volumes generally produced by members of this clade.

We suggest that flower and inflorescence evolution have been jointly shaped by the need to optimize long-distance visibility to pollinators (competition with the surrounding vegetation) and protect the nectar against desiccation. This implies that these adaptations fine-tune the avian pollination syndrome to suit the ecology of the region.

Speciation—The apparent lack of pollinator specialization in *Melianthus* implies that the mechanisms underlying the genetic isolation associated with speciation have to be sought elsewhere. The highly restricted ranges of most species argues for an important role for allopatry, with climatic and edaphic factors playing a key role in selecting for novel morphologies.

Melianthus major and *M. villosus* occur in the wettest habitats of any *Melianthus* species, the former being associated with ground water and the latter with the wet Drakensberg Escarpment. Despite ages of 19 and 17 My, respectively, there has been no diversification in these two lineages. At least in the Cape flora, species-poor lineages are largely restricted to the wettest habitats, and these lineages are often regarded as relicts of previously more widespread clades (Levyns, 1962; Goldblatt, 1997; Linder, 2003). However, these are also the geographically most restricted habitats and the interpretation that the small number of species is the result of physical space limitation cannot be rejected.

In *Melianthus* two radiations are evident in drier environments, both along the escarpment mountains, in what are topographically complex areas. Increased speciation in mountainous areas has already been demonstrated for the Americas (von Hagen and Kadereit, 2003). In the case of the eastern clade, at least, speciation seems to have been associated with uplift events during the Miocene and Pliocene, these generating a series of sharp rainfall gradients on the eastern escarpment. By contrast, the emergence of the western clade was associated with adaptation to a winter rainfall regime. The species comprising both lineages are allopatric and in both lineages show some evidence of edaphic specialization. In the western lineage, *M. elongatus* has specialized to coastal Quaternary sands (Fig. 1h), whereas in the eastern clade, *M. comosus* is largely found on alluvial soils derived from shales. Speciation thus appears to have involved a combination of allopatry, edaphic specialization, and shifts in total rainfall. Such combinations have been repeatedly documented for diverse families in the Cape flora (Goldblatt, 1982; Kurzweil et al., 1991; Linder and Hardy, 2005), but appear not to have been documented for the general southern African flora.

The low diversification rate in *Melianthus*, compared to many other southern African clades of a similar age (Klak et al., 2004; Linder and Hardy, 2004), is curious. We suggest that this could be the result of the unspecialized bird pollination system. Although bird pollination is fairly common in the southern African flora (Rebelo, 1987), it is generally a derived syndrome, found in a few species in mostly insect-pollinated lineages (e.g., Johnson, 1996; e.g., Manning and Goldblatt, 2005). There has as yet been no investigation of the consequences to the speciation rate of a change to bird pollination. It is striking, though, that where the pollinators are known, biotically pollinated lineages with high diversification rates tend to have a remarkably broad range of pollination syndromes (Johnson et al., 1998; Goldblatt et al., 2002; Manning and Goldblatt, 2005, 2006), suggesting that being

"locked" into a single pollination system might lead to lower diversification rates. Furthermore, bird pollination might result in longer gene-flow distances, thus inhibiting speciation.

It remains unclear whether the changes in *Melianthus* were in response to changing conditions or whether it gradually evolved the ability to deal with existing conditions. Paleoecological reconstructions suggest the former: in the middle Miocene southern Africa lacked dry, and most certainly summer-dry, habitats. Then the inferred ecological evolution in *Melianthus* reflects the climatic evolution of the subcontinent. If this is the case, we would expect many other taxa to have similar patterns, possibly reflecting a general model for the diversification of the southern African flora.

LITERATURE CITED

AGARWAL, J. S., AND R. P. RASTOGI. 1976. Queretaroic (30) caffeate and other constituents of *Melianthus major*. *Phytochemistry* 15: 430–431.

ANDERSON, L. A. 1968. Occurrence of oleanolic acid acetate and ellagic acid in *Melianthus comosus* Vahl. *Journal of the South African Chemical Institute* 21: 91–92.

ANDERSON, L. A., AND J. M. KOEKEMOER. 1968. Chemistry of *Melianthus comosus* Vahl. 2. Isolation of hellebrigenin 3-acetate and other unidentified bufadienolides from rootbark of *Melianthus comosus* Vahl. *Journal of the South African Chemical Institute* 21: 155–159.

ANDERSON, L. A., AND J. M. KOEKEMOER. 1969. Chemistry of *Melianthus comosus* Vahl. 3. Constitution of melianthugenin and melianthusigenin, new bufadienolides from root-bark. *Journal of the South African Chemical Institute* 22: 191–197.

ANDREWS, S. 1987. *Melianthus villosus*. *Kew Magazine* 4: 123–127.

APG. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141: 399–436.

ARCHIBALD, J. K., M. E. MORT, AND A. D. WOLFE. 2005. Phylogenetic relationships within *Zaluzianskya* (Scrophulariaceae s.s., tribe Manuleeae): classification based on DNA sequences from multiple genomes and implications for character evolution and biogeography. *Systematic Botany* 30: 201–215.

AXELROD, D. I., AND P. H. RAVEN. 1978. Late Cretaceous and Tertiary vegetation history of Africa. *In* M. J. A. Werger [ed.], Biogeography and ecology of southern Africa, 79–130. Junk, The Hague, Netherlands.

BAKKER, F. T., A. CULHAM, E. MARAIS, AND M. GIBBY. 2005. Nested radiation in Cape *Pelargonium*. *In* F. T. Bakker, L. W. Chatrou, B. Gravendeel, and P. B. Pelser [eds.], Plant species-level systematics: new perspectives on pattern & process, 75–100. Koeltz, Königstein, Germany.

BELL, C. D., AND M. J. DONOGHUE. 2005. Dating the Dipsacales: comparing models, genes, and evolutionary implications. *American Journal of Botany* 92: 284–296.

BUCKLER, E., A. IPPOLITO, AND T. HOLTSFORD. 1997. The evolution of ribosomal DNA: paralogues and phylogenetic implications. *Genetics* 145: 821–832.

BURTT DAVEY, J. 1932. A manual of flowering plants and ferns of the Transvaal and Swaziland. Longmans, London, UK.

CONTI, E., F. RUTSCHMANN, T. ERIKSSON, K. J. SYTSMA, AND D. A. BAUM. 2004. Calibration of molecular clocks and the biogeographic history of Crypteroniaceae: A reply to Moyle. *Evolution* 58: 1874–1876.

DAHLGREN, R. M., AND A. E. VAN WYK. 1988. Structures and relationships of families endemic to or centered in southern Africa. *Monogaphs of Systematic Botany from the Missouri Botanical Garden* 25: 1–94.

DOWELD, A. B. 2001. The systematic relevance of fruit and seed structure in *Bersama* and *Melianthus* (Melianthaceae). *Plant Systematics and Evolution* 227: 75–103.

ERDTMAN, G. 1952. Pollen morphology and plant taxonomy. Almqvist & Wiksell, Stockholm, Sweden.

FAEGRI, K., AND L. VAN DER PIJL. 1979. The principles of pollination biology. Pergamon Press, Oxford, UK.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FITCH, W. M. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. *Systematic Zoology* 20: 406–416.

GAWEL, N., AND R. JARRETT. 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Plant Molecular Biology Reporter* 9: 262–266.

GERMISHUIZEN, G., AND N. L. MEYER. 2003. Plants of southern Africa: an annotated checklist. National Botanical Institute, Pretoria, South Africa.

GOLDBLATT, P. 1982. Systematics of *Freesia* Klatt (Iridaceae). *Journal of South African Botany* 48: 39–91.

GOLDBLATT, P. 1997. Floristic diversity in the Cape Flora of South Africa. *Biodiversity and Conservation* 6: 359–377.

GOLDBLATT, P., AND J. C. MANNING. 2002. Plant diversity of the Cape Region of southern Africa. *Annals of the Missouri Botanical Garden* 89: 281–302.

GOLDBLATT, P. 2006. Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. *Annals of Botany* 97: 317–344.

GOLDBLATT, P., V. SAVOLAINEN, O. PORTEOUS, I. SOSTARIC, M. POWELL, G. REEVES, J. C. MANNING, T. G. BARRACLOUGH, AND M. W. CHASE. 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution* 25: 341–360.

GRAUR, D., AND W. MARTIN. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics* 20: 80–86.

GRIEBELER, E. M. 2004. Gammastatistic, version 1.0. Institut für Zoologie, Abteilung Ökologie, Johannes Gutenberg-Universität, Mainz, Germany.

HARDY, C. R., AND H. P. LINDER. 2005. Reconstructing ancestral habitats and ecologies: accounting for intraspecific variability and issues of timing in ecological diversification. *Systematic Biology* 54: 299–316.

HEADS, M. 2005. Dating nodes on molecular phylogenies: a critique of molecular biogeography. *Cladistics* 21: 62–78.

HEDGES, S. B., AND S. KUMAR. 2004. Precision of molecular time estimates. *Trends in Genetics* 20: 242–247.

HERSHKOVITZ, M., AND L. LEWIS. 1996. Deep level diagnostic value of the rDNA-ITS region: the case of an algal interloper. *Molecular Biology and Evolution* 13: 167–177.

HOLMGREN, P. K., AND N. H. HOLMGREN. 1998, onwards. Index Herbariorum. New York Botanical Garden. Website http://sciweb.nybg.org/science2/IndexHerbariorum.asp [Accessed 18 April 2006].

JOHNSON, S. D. 1996. Bird pollination in South African species of *Satyrium* (Orchidaceae). *Plant Systematics and Evolution* 203: 91–98.

JOHNSON, S. D. 1996. Pollination, adaptation and speciation models in the Cape flora of South Africa. *Taxon* 45: 59–66.

JOHNSON, S. D., AND K. E. STEINER. 2000. Generalization versus specialization in plant pollination systems. *Trends in Evolution and Ecology* 15: 140–143.

JOHNSON, S. D., AND K. E. STEINER. 2003. Specialized pollination systems in southern Africa. *South African Journal of Science* 99: 345–348.

JOHNSON, S. D., H. P. LINDER, AND K. E. STEINER. 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany* 85: 402–411.

KELMANSON, J. E., A. K. JAGER, AND J. VAN STADEN. 2000. Zulu medicinal plants with antibacterial activity. *Journal of Ethnopharmacology* 69: 241–246.

KLAK, C., G. REEVES, AND T. A. HEDDERSON. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature* 427: 63–65.

KOEKEMOER, J. M., L. A. ANDERSON, AND K. G. R. PACHLER. 1970. Chemistry of *Melianthus comosus* Vahl. Part IV. 6-β-acetoxymelianthugenin, a new toxic principle from root-bark. *Journal of the South African Chemical Institute* 23: 146–153.

KOEKEMOER, J. M., L. A. ANDERSON, AND K. G. R. PACHLER. 1971. Chemistry of *Melianthus comosus* Vahl. 5. 2 Novel bufadienolides from root-bark. *Journal of the South African Chemical Institute* 24: 75–86.

KOEKEMOER, J., N. M. J. VERMEULEN, AND L. A. ANDERSON. 1974. Chemistry of *Melianthus comosus* Vahl. 6. Structure of a new triterpenoid acid from root-bark. *Journal of the South African Chemical Institute* 27: 131–136.

KURZWEIL, H., H. P. LINDER, AND P. CHESSELET. 1991. The phylogeny and evolution of the *Pterygodium–Corycium* complex (Coryciinae, Orchidaceae). *Plant Systematics and Evolution* 175: 161–223.

LEOPOLD, E. B., G. LIU, AND S. CLAY-POOLE. 1992. Low-biomass vegetation in the Oligocene? *In* D. R. Prothero and W. A. Berggren [eds.], Eocene-Oligocene climatic and biotic evolution, 399–436. Princeton University Press, Princeton, New Jersey, USA.

LEVYNS, M. R. 1962. Possible antarctic elements in the South African Flora. *South African Journal of Science* 58: 237–241.

LINDER, H. P. 2003. The radiation of the Cape flora, southern Africa. *Biological Reviews* 78: 597–638.

LINDER, H. P. 2005. The evolution of diversity: the Cape flora. *Trends in Plant Science* 10: 536–541.

LINDER, H. P., AND D. M. MANN. 1998. The phylogeny and biogeography of *Thamnochortus* (Restionaceae). *Botanical Journal of the Linnean Society* 128: 319–357.

LINDER, H. P., AND C. R. HARDY. 2004. Evolution of the species-rich Cape flora. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 359: 1623–1632.

LINDER, H. P., AND C. R. HARDY. 2005. Species richness in the Cape flora: a macroevolutionary and macroecological perspective. *In* F. T. Bakker, L. W. Chatrou, B. Gravendeel, and P. B. Pelser [eds.], Plant species-level systematics: new perspectives on pattern & process. 47–73. Koeltz, Königstein, Germany.

LINDER, H. P., C. R. HARDY, AND F. RUTSCHMANN. 2005. Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Molecular Phylogenetics and Evolution* 35: 569–582.

LINDER, H. P., J. C. LOVETT, J. MUTKE, W. BARTHLOTT, N. JÜRGENS, A. G. REBELO, AND W. KÜPER. 2005. A numerical re-evaluation of the sub-Saharan phytochoria of mainland Africa. *Biologist Skrifter* 55: 229–252.

MACLEAN, G. 1993. Roberts' birds of southern Africa. John Voelcker Bird Book Fund, Cape Town, South Africa.

MANNING, J. C., AND H. P. LINDER. 1992. Pollinators and evolution in *Disperis* (Orchidaceae), or why are there so many species? *South African Journal of Science* 88: 38–49.

MANNING, J. C., AND P. GOLDBLATT. 2005. Radiation of pollination systems in the Cape genus *Tritoniopsis* (Iridaceae: Crocoideae) and the development of bimodal pollination strategies. *International Journal of Plant Sciences* 166: 459–474.

MARLOTH, R. 1908. Some observations on entomophilous flowers. *South African Journal of Science* 4: 110–113.

MARLOTH, R. 1925. Flora of South Africa, vol. 2, section 2. Darter, Cape Town, South Africa.

MUMMENHOFF, K., I. A. AL-SHEHBAZ, F. T. BAKKER, H. P. LINDER, AND A. MÜHLHAUSEN. 2005. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of Southern Africa. *Annals of the Missouri Botanical Garden* 92: 400–424.

PARTRIDGE, T. C., AND R. R. MAUD. 2000. Macro-scale geomorphic evolution of southern Africa. *In* T. C. Partridge and R. R. Maud [eds.], The Cenozoic of southern Africa, 3–18. Oxford University Press, Oxford, UK.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

PROCTOR, M., P. YEO, AND A. LACK. 1996. The natural history of pollination. Harper Collins, London, UK.

REBELO, A. G. 1987. Bird pollination in the Cape flora. *In* A. G. Rebelo [ed.], A preliminary synthesis of pollination biology in the Cape flora, 83–108. South African National Scientific Programmes Report, Pretoria, South Africa.

RONSE DECRAENE, L. P., AND E. F. SMETS. 1999. Similarities in floral ontogeny and anatomy between the genera *Francoa* (Francoaceae) and *Greyia* (Greyiaceae). *International Journal of Plant Sciences* 160: 377–393.

RONSE DECRAENE, L. P., H. P. LINDER, T. DLAMINI, AND E. F. SMETS. 2001. Evolution and development of floral diversity of Melianthaceae, an enigmatic family from Southern Africa. *International Journal of Plant Sciences* 162: 59–82.

ROTH, I. 1949. Zur Entwicklungsgeschichte des Blattes, mit besonderer Berücksichtigung von Stipular- und Ligularbildungen. *Planta* 37: 299–336.

RUTSCHMANN, F. 2004. Bayesian molecular dating using PAML/multidivtime. A step-by-step manual. University of Zurich, Zurich, Switzerland.

SANDERSON, M. J. 1990. Estimating rates of speciation and evolution: a bias due to homoplasy. *Cladistics* 6: 387–391.

SANG, T., D. J. CRAWFORD, AND T. STUESSY. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using sequences of internal transcribed spacer of nuclear ribosomal DNA; implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences, USA* 92: 6813–6817.

SAVOLAINEN, V., M. F. FAY, D. C. ALBACH, A. BACKLUND, M. VAN DER BANK, K. M. CAMERON, S. A. JOHNSON, M. D. LLEDO, J.-L. PINTAUD, M. POWELL, M. C. SHEAHAN, D. E. SOLTIS, P. S. SOLTIS, P. WESTON, AND M. W. CHASE. 2000. Phylogeny of the eudicots: a nearly complete familial analysis based on *rbcL* gene sequences. *Kew Bulletin* 55: 257–309.

SCHULZE, R. E. 1997. South African atlas of agrohydrology and climatology. Water Research Commission, Report TT82/96, Pretoria, South Africa.

SCHULZE, R. E. 1997. Climate. *In* R. M. Cowling, D. M. Richardson, and S. M. Pierce [eds.], Vegetation of southern Africa. Cambridge University Press, Cambridge, UK.

SCOTT-ELLIOT, F. 1890. Ornithophilous plants in South Africa. *Annals of Botany* 4: 265–280.

SCOTT, L. 1999. Vegetation history and climate in the savanna biome South Africa since 190,000 ka: a comparison of pollen data from the Tswaing Crater (the Pretoria Saltpan) and Wonderkrater. *Quaternary International* 57–58: 215–223.

SCOTT, L., AND J. C. VOGEL. 2000. Evidence for environmental conditions during the last 20 000 years in southern Africa from C-13 in fossil hyrax dung. *Global and Planetary Change* 26: 207–215.

SCOTT, L., H. M. ANDERSON, AND J. M. ANDERSON. 1997. Vegetation history. *In* R. M. Cowling, D. M. Richardson, and S. M. Pierce [eds.], Vegetation of southern Africa, 62–84. Cambridge University Press, Cambridge, UK.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

SKEAD, C. 1967. Sunbirds of southern Africa. Cape and Transvaal Printers, Cape Town, South Africa.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PATOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

THORNE, J. L., AND H. KISHINO. 2002. Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology* 51: 689–702.

VAN WYK, A. E., AND G. F. SMITH. 2001. Regions of floristic endemism in Southern Africa. Umdaus Press, Hatfield, South Africa.

VERBOOM, G. A., H. P. LINDER, AND W. D. STOCK. 2003. Phylogenetics of the grass genus *Ehrharta* Thunb.: evidence for radiation in the summer-arid zone of the South African Cape. *Evolution* 57: 1008–1021.

VERBOOM, G. A., H. P. LINDER, AND W. D. STOCK. 2004. Testing the adaptive nature of radiation: growth form and life history divergence in the African grass genus *Ehrharta* (Poaceae: Ehrhartoideae). *American Journal of Botany* 91: 1364–1370.

VERDCOURT, B. 1950. Notes on the genus *Bersama* in Africa. *Kew Bulletin* 1950: 233–244.

VOGEL, S. 1954. Blütenbiologische Typen als elemente der Sippengliederung. Gustav Fisher Verlag, Jena, Germany.

VON HAGEN, K. B., AND J. W. KADEREIT. 2003. The diversification of *Halenia* (Gentianaceae): Ecological opportunity versus key innovation. *Evolution* 57: 2507–2518.

VON MARILAUN, A. 1895. The natural history of plants. Blackie and Sons, Glasgow, UK.

WASER, N. M. 1998. Pollination, angiosperm speciation, and the nature of species boundaries. *Oikos* 81: 198–201.

WATT, J. M., AND M. G. BREYER-BRANDWIJK. 1962. The medicinal and poisonous plants of southern and eastern Africa. E. & S. Livingstone, Edinburgh, UK.

WHITE, T. J., T. D. BRUNS, S. B. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of fungal RNS genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications, 315–322. Academic Press, San Diego, California, USA.

WIKSTRÖM, N., V. SAVOLAINEN, AND M. W. CHASE. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London, B, Biological Sciences* 268: 2211–2220.

YANG, Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. *CABIOS (Computer Applications in the Biosciences)* 13: 555–556.

YOUNG, N. D., AND J. HEALY. 2003. GapCoder automates the use of indel characters in phylogenetic analysis. BMC Bioinformatics 4:6 doi: 10.1186/1471-2105-4-6.

ZACHOS, J., M. PAGANI, L. SLOAN, E. THOMAS, AND K. BILLUPS. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292: 686–693.

APPENDIX. Voucher information and GenBank accession numbers for ITS, *trnL-trnF*, and *psbA* sequences from taxa used in this study. Voucher specimens are deposited in the Bolus Herbarium of the University of Cape Town.

***Taxon*:** GenBank accession nos. ITS, *trnL-trnF*, *psbA*; *Source* (Acc. no.)

Bersama lucens (Hochst.) Szyszyl.: DQ435401, DQ435381, DQ435421; Cult. Kirstenbosch Bot. Gard. (439), *J. Henning 34*. ***B. swinnyi*** Phillips, DQ435402, DQ435382, DQ435422; Cult. Kirstenbosch Bot. Gard. (441), *J. Henning 35*.

Greyia flanaganii Bolus, DQ435399, DQ435379, DQ435419; Cult. Kirstenbosch Bot. Gard. (444), *J. Henning 32*. ***G. radlkoferi*** Szyszyl., DQ435400, DQ435380, DQ435420; Cult. Kirstenbosch Bot. Gard. (203), *J. Henning 31*.

Melianthus comosus Vahl 1, DQ435407, DQ435387, DQ435427; Namibia, Rosh Pinah, *J. Henning 3*. ***M. comosus*** Vahl 2, DQ435408, DQ435388, DQ435428; South Africa, Nieuwoudtville, *J. Henning 34*. ***M. comosus*** Vahl 3, DQ435409, DQ435389, DQ435429; South Africa, Montagu, *J. Henning 21*. ***M. comosus*** Vahl 4, DQ435410, DQ435390, DQ435430; South Africa, Golden Gate, *J. Henning 25*. ***M. comosus*** Vahl 5, DQ435411, DQ435391, DQ435431; South Africa, Zastron, *J. Henning 27*. ***M. elongatus*** Wijn., DQ435418, DQ435398, DQ435438; South Africa, Velddrif, *J. Henning 2*. ***M. insignis*** (Phill. & Hoff.) Tansley and Schelpe, DQ435413, DQ435393, DQ435433; South Africa, Golden Gate, *J. Henning 24*. ***M. dregeanus*** (Sond.) Tansley and Schelpe, DQ435412, DQ435392, DQ435432; South Africa, Cathcart, *J. Henning 29*. ***M. major*** L. 1, DQ435403, DQ435383, DQ435423; South Africa, Montagu, *J. Henning 1*. ***M. major*** L. 2, DQ435404, DQ435384, DQ435424; South Africa, Cape Peninsula, *J. Henning 20*. ***M. major*** L. 3, DQ435405, DQ435385, DQ435425; South Africa, Citrusdal, *J. Henning 16*. ***M. pectinatus*** (Harv.) Tansley and Schelpe 1, DQ435414, DQ435394, DQ435434; South Africa, Steinkopf, *J. Henning 9*. ***M. pectinatus*** (Harv.) Tansley and Schelpe 2, DQ435415, DQ435395, DQ435435; South Africa, Kamiesberg, *J. Henning 13*. ***M. gariepinus*** (Merxm. & Roess.) Tansley and Schelpe 1, DQ435416, DQ435396, DQ435436; Namibia, Rosh Pinah, *J. Henning 6*. ***M. gariepinus*** (Merxm. & Roess.) Tansley and Schelpe 2, DQ435417, DQ435397, DQ435437; Namibia, Rosh Pinah, *J. Henning 4*. ***M. villosus*** Bolus, DQ435406, DQ435386, DQ435426; Cult. Kirstenbosch Bot. Gard. (857), *J. Henning 33*.