Evolution of the species-rich Cape flora

H. P. Linder* and C. R. Hardy

Institute of Systematic Botany, Zollikerstrasse 107, CH-8008 Zurich, Switzerland

The Cape Floristic Region ('fynbos biome') has very high levels of plant species diversity and endemism. Much of this diversity is concentrated in a relatively small number of clades centered in the region (Cape clades), and these form a vegetation called 'fynbos'. The general explanation for the origin of this diversity is that much of it evolved in the Pliocene and Late Miocene in response to progressive aridification. We present a phylogenetic analysis of an almost complete species sample of the largest clade of Restionaceae, the third largest Cape clade. This indicates that the radiation of the Restionaceae started between 20 and 42 Myr ago, and since then there were no, or at most gradual, changes in the speciation rate in this clade. For seven other clades, the estimated starting dates for their radiation ranges from 7 to 20 Myr ago. Combining the radiation patterns for these clades shows that *ca.* 15% of the modern species evolved during the Pleistocene, and almost 40% since the beginning of the Pliocene. We suggest that these clades might have radiated in response to the fynbos vegetation increasing its extent in the Cape as a result of climatic change.

Keywords: Cape flora; evolutionary radiation; diversification rate; molecular clocks; Restionaceae; non-parametric rate smoothing

1. INTRODUCTION

The generation of large species flocks holds a general fascination, and several papers have recently attempted to set records with recent and rapid speciation (Turner 1999; Richardson *et al.* 2001a,b; Verheyen *et al.* 2003; Klak *et al.* 2004). The southern tip of Africa contains a number of plant lineages that are thought to have undergone recent and rapid speciation, and it could be argued that the whole flora might be the result of such a recent burst of speciation (Linder *et al.* 1992). Indeed, two botanical examples of recent and rapid speciation (Richardson *et al.* 2001b; Klak *et al.* 2004) do come from the southern tip of Africa.

The flora of the southern tip of Africa is unusually rich, and contains at least two remarkable centres of diversification: the CFR and the Succulent Karoo Region (Van Wyk & Smith 2001). Of these, the CFR is the richer, with *ca.* 9000 species in an area of 90 000 km², and with 68.8% endemism at species level (Goldblatt & Manning 2002). On the basis of this very high species richness and endemism it has long been recognized as a floristic region or a regional centre of endemism (Bulus 1886; Marloth 1908; Goldblatt 1978; White 1983), and on the basis of the number of endemic families and genera, even as a floristic kingdom (Good 1974; Takhtajan 1986). Much of this richness is concentrated in few large clades, which most probably originated and radiated within the CFR, with outlying species of many of these clades now found in the cool upland areas of tropical Africa, rarely reaching beyond the Sahara and Arabian deserts. A recent tabulation indicated that 50% of the flora is contained in the 30 largest

clades (Linder 2003). The taxonomic expression of this is the dominance of the flora by a few large genera, of which *Erica*, with its 658 species, is the most extreme (Goldblatt & Manning 2000).

The most popular reconstruction of the evolutionary history of this flora (Levyns 1964; Linder *et al.* 1992; Goldblatt & Manning 2000; Linder 2003) can be simplified as follows. The Mid-Miocene vegetation (and flora) of the area was tropical, possibly with the ancestral lineages of the modern Cape flora restricted to the mountains. The mountains are built of erosion-resistant sandstones, which result in oligotrophic soils and could consequently have sustained a heathy vegetation. The climate was tropical or subtropical and mesic, with no dry season. With the steepening of the global pole-to-equator climatic gradients and the complete glaciation of Antarctica, the upwelling of cold bottom waters along the Atlantic seaboard of southern Africa, which started some 8–10 Myr ago (Siesser 1980), resulted in conditions that blocked off the summer rainfall, leaving only the winter rainfall and, thus, causing the modern climatic system. This led to the extinction of the tropical flora, opening vacant habitat into which the lineages of heathy plants from the mountains then radiated, generating the modern species flocks of the more or less 30 most successful clades of Cape plants. The dating for this radiation was considered to have been in the Neogene: Goldblatt (1997) suggested that the Cape flora could not be distinguished before the Pliocene, less than 5 Myr ago, while Cowling & Pressey (2001) dated the diversification to the Late Pliocene. This process may have been facilitated by regular fires in the hot, dry summer periods.

The fossil record of the CFR has been very poorly investigated, although some potentially good fossiliferous areas might exist. The best known period is the Holocene, while older deposits are scarce or unknown. The Neogene

* Author for correspondence (plinder@systbot.unizh.ch).

One contribution of 16 to a Discussion Meeting Issue 'Plant phylogeny and the origin of major biomes'.

is known from only a few deposits, and the dating control on these is weak (for a critical review see Linder 2003). Consequently, the hypothesis presented above has to be tested against the patterns in the extant flora, as the available fossil data are too sparse to allow a rigorous testing. There have been a number of molecular clock-based estimates of the initiation of the radiation of several Cape clades. These are consistent with a broader Miocene initiation of the radiation in the flora. The ages range from 7 Myr ago for *Phyllica* (Richardson *et al.* 2001b) to 20–15 Myr ago for Iridaceae (Reeves *et al.* 2001; Goldblatt *et al.* 2002).

Here, we use a detailed investigation of the pattern of lineage diversification in the largest clade of African Restionaceae, involving 289 species, to test several aspects of the evolutionary scenario for the Cape flora presented above. Specifically, we wish to determine whether the initiation date agrees with the Late Miocene date implied by the Levyns–Goldblatt hypothesis, as well as the *Phyllica* data. Furthermore, we would like to know whether the radiation rate in Restionaceae has slowed, or is still continuing at the same rate as it started. We will place this in context with the other available information on the diversification of the Cape flora, and seek a general pattern in the relationship between lineage diversification, palaeoenvironmental change, and the evolution of the vegetation in the fynbos biome.

2. MATERIAL AND METHODS

(a) *Topology of Restionaceae*

The study group is the ‘*Restio*-clade’ of the African Restionaceae, which includes 289 of the 350 species of African Restionaceae. The African Restionaceae constitute the third largest clade of the Cape flora (Linder 2003), and are ecologically dominant over large areas of ‘fynbos’ vegetation, the vegetation type most associated with the Cape floral elements (Taylor 1978). The ‘*Restio*-clade’ is almost restricted to the CFR, with only six species widespread in southern and south-central Africa and Madagascar. Eldenäs & Linder (2000) demonstrated strong molecular support for the monophyly of the ‘*Restio*-clade’, even though there is little or no morphological support for its monophyly (Linder 1984). In the current analysis, 265 species (i.e. 92%) of this clade were included. Additionally, two accessions of the variable and widespread species *Ischyrolepis macer* and both subspecies of *Restio dodii* were included to give a sampling of 267 plants, representing 265 species of the ‘*Restio*-clade’. Out of the 24 species that were not included, the sequencing of five is in progress, three are possibly not taxonomically distinct (for detailed comment see Linder 2001), and the remainder could not be located either because of their inaccessible habitat (not enough field time) or simply because we could not find them. Attempts to extract DNA from herbarium material met with only limited success.

Based on the phylogenetic results of Eldenäs & Linder (2000) and Linder *et al.* (2003), an outgroup comprising 24 terminals representing 23 out of 61 species of the African ‘*Willdenowia*-clade’ was included in the analysis. The cladograms resulting from our analysis were rooted with a single terminal representing *Baloskion tetraphyllum*, a species of the Australian Restionaceae, a group shown by Briggs *et al.* (2000) and Linder *et al.* (2003) to not be a part of the African Restionaceae.

DNA sequences were generated from the plastid regions spanning the *trnL* intron and the *trnL*–*trnF* intergenic spacer (Taberlet

et al. 1991), the complete gene encoding *rbcl* (Chase & Albert 1998), the complete *atpB*–*rbcl* intergenic spacer (Manen *et al.* 1994; Chiang & Schaal 2000; Cuénoud *et al.* 2000), and *matK* plus the flanking *trnK* intron (Hilu & Liang 1997). Total DNA was isolated from silica gel-dried culms using the Dneasy Plant Mini Kit (Qiagen Inc., Valencia, CA). Sequences were generated using standard methods for PCR amplification and automated sequencing.

Raw sequence data files were analysed with the ABI PRISM 377 Software Collection, v. 2.1. Contigs were constructed in SEQUENCHER and alignments were performed using the default alignment parameters in CLUSTAL-X (Thompson *et al.* 1997), followed by adjustment by eye. These sequences were assembled into a single matrix in WINCLADA (Nixon 2002). All characters were weighted equally and treated as non-additive during tree searches. Indels were not coded as characters, as these could not be used in branch length estimates. Accurate grouping of indels into characters proved to be difficult, and inclusion of indels did not significantly impact the cladogram topology. This data matrix has been deposited at www.treebase.org.

Parsimony searches were conducted using the parsimony ratchet (Nixon 1999) as implemented in WINCLADA, running NONA v. 1.6 (Goloboff 1993) as a daughter process. Ten ratchet searches were conducted, each initiated with the generation of a Wagner tree, using a random taxon entry sequence, followed by TBR branch swapping with one tree retained and used as the starting point for 500 ratchet cycles. In the weighted and constrained half of each ratchet cycle, a randomly selected set of 10% of the characters were resampled, and a randomly selected set of 10% of the resolved clades were constrained.

Bootstrap support values (Felsenstein 1985) were obtained using NONA spawned as a daughter process in WINCLADA using 815 replicates with 50 TBR searches each, holding one tree per TBR search. Percentages were then based on the strict consensus tree of each of the 815 replicates.

(b) *Branch length estimation*

We used MODELTEST v. 3.06 (Posada & Crandall 1998) in tandem with PAUP* v. 4.0 (Swofford 2002) to find the least complicated of the possible 56 models of sequence evolution that is not significantly different by the likelihood ratio test (Felsenstein 1981) from the next-most complicated model. As an input tree, the MODELTEST default of a neighbour-joining tree was used. The optimal model returned was a general time reversible model, with invariable sites and among-site rate heterogeneity, modelled using a discrete approximation of a gamma distribution. Using this model (GTR + I + G), the log-likelihoods of a clock-constrained and an unconstrained tree, for two randomly selected trees from the set of MPTs, were compared using the likelihood ratio test. In both cases, the clock was rejected (e.g. the tree in figure 1: $\chi^2 = 492.2$, d.f. = 290, $p < 0.05$). Thus, branch lengths of these two, as well as an additional 98 randomly selected trees from the set of MPTs, were estimated using the GTR + I + G model in PAUP*.

The branch lengths obtained by the maximum likelihood analysis were made ultrametric using NPRS (Sanderson 1997), as implemented in TREEEDIT (Rambaut & Charleston 2004). A penalized likelihood analysis was also desired, but our attempts to find an optimal rate-smoothing value with the prerequisite cross-validation procedure (Sanderson 2002) were unsuccessful, possibly because of the large dataset. Robustness of the results obtained from NPRS to different MPT topologies was tested by

Figure 1. Phylogram of one of the most parsimonious trees, indicating the outgroup and the study group (in brackets). The branch lengths are calculated using maximum likelihood, the model is specified in the text. Rate classes assigned to particular clades and their subtending branches are designated by black rectangles. The fifth rate class (the default rate class '0') was assigned to every other branch in the tree. The black dot marks the calibration node.

applying the procedure to the 100 MPTs for which maximum likelihood branch lengths were estimated.

Additionally, we estimated the ultrametric tree using a global clock in PAUP*, and local clocks in PAML, v. 3.14 (Yang 1997). For the local clocks analysis, five rate classes were chosen based upon a visual inspection of the phylogram depicted in figure 1. These classes were assigned to clades (and their subtending branches) with noticeably different rates of molecular evolution than other clades (Yoder & Yang 2000). Having designated the appropriate clades that were to be assigned distinct rate classes in the tree file, the ultrametric tree was estimated using the program 'BASEML' of the PAML package, under the GTR + G model of nucleotide substitution (using a discrete approximation of a gamma distribution with four rate categories and shape parameter estimated in BASEML). The direct estimation of a proportion of invariant sites (I), as called for under the GTR + G + I model selected by MODELTEST, is not available in PAML.

(c) Calibration

Three sets of calibrations were taken into consideration to transform the relative time tree into an absolute time tree. The first used the dates from Linder *et al.* (2003), which are based on a 61–64 Myr old fossil deposit of Restionaceae pollen from Banke, South Africa (Scholtz 1985). This sets a secondary date of 49.8 Myr (a minimum of 43.7 Myr, a maximum of 55.9 Myr) for

the separation between the *Willdenowia* and the *Restio*-clade of the African Restionaceae, with NPRS transformation (Linder *et al.* 2003). We simplified the analysis by using only the mean date for the subsequent analyses, and ignoring the ranges. The second calibration is from Bremer (2002), which itself was based on several calibration points. However, this calibration could not be implemented, because the interior node used here was not included in their sampling. The third calibration is from Wikström *et al.* (2001), but their dates on terminal nodes are often very recent by comparison with fossil data, an artefact that may be the result of undersampling the terminal lineages (Wikström *et al.* 2004). As Wikström *et al.* dates were used for some other analyses as secondary dating points, we included these in our calculations as well.

Based on these calibrations, the absolute node ages and ultrametric branch lengths obtained for the various trees and clock methods were extracted from their respective output files and plotted against the accumulation of species in SPSS, v. 12.0, to construct an LTT plot (Nee *et al.* 1992). Changes in speciation rate were evaluated for 10 time periods (2 Myr intervals from the present to 14 Myr ago, then to 20 Myr ago, and finally more than 30 Myr ago) with an ANOVA analysis (least significant difference *post hoc* test), as well as the Student's *t*-test comparing adjacent time intervals, as implemented in SPSS.

3. RESULTS

The combined molecular data matrix consisted of 6854 aligned bases, of which there were 1529 parsimony-informative sites. The ratchet analyses located 2405 most parsimonious trees with a length of 4787, a consistency index of 0.44 and a retention index of 0.85. The strict consensus tree is largely resolved, with only 59 out of the 290 nodes polytomous. Out of the total of 290 African Restionaceae internal nodes displayed in a randomly selected MPT (figure 1), 175 (60%) have bootstrap support in excess of 70%, and 132 (46%) in excess of 90%. Within the *Restio*-clade, 162 (60%) have bootstrap support in excess of 70%, and 120 (46%) in excess of 90%.

The phylograms of the 100 randomly selected MPTs have similar branch lengths (e.g. figure 1), and this is reflected by the fact that the NPRS of these resulted in similar LTT plots. Because of the long computing times required for this large dataset, the global and local clocks analyses were conducted only on the single MPT illustrated in figure 1. We assume that our inferences on speciation rates based on this tree would be robust to alternative MPT topologies, based on the following observations: (i) the phylograms of each of the 100 randomly chosen MPTs are similar in their branch lengths, and (ii) NPRS of these trees results in ultrametric trees and LTT plots that are not qualitatively different from one another (figure 2).

These analyses indicate that the *Restio*-clade radiation started 28 Myr ago (local clocks), 30.19 Myr ago (global clock), or 42.83 Myr ago (NPRS) (table 1), the latter with a standard deviation of 0.28 Myr ($n = 50$; figure 3). The NPRS analyses all depict a significant slowing of the radiation towards the present, the global clock analysis depicts a more or less constant radiation rate, and the local clocks analysis depicts a gradual acceleration in the radiation towards the present.

Figure 2. The influence of most parsimonious tree topology variation on NPRS LTT plots. Shown here are 50 of the 100 randomly selected MPTs. The thick black line is the curve derived from figure 1. The time-scale here is based on the 49.8 Myr average age for the *Restio*-clade determined from Linder *et al.* (2003).

Table 1. Approximate times of the initiation of the radiation of Restionaceae, in millions of years ago, illustrating the divergent results obtained by the different calibrations used, and the various rate correction methods employed.

	NPRS (TREEDIT)	global clock (PAUP)	local clock (PAML)
pollen (using secondary date from Linder <i>et al.</i> (2003))	42.83	30.19	28
Wikström (2001; approximate tertiary date to estimate interior node)	29	22	20

Although there is generally variation in the speciation rate through time, much of it is not significantly different (table 2). In the NPRS analysis (figure 4a), no temporally adjacent rates show significant variation, but the rates at the beginning of the time period are significantly different from the rates towards the end of the time period. In the global clock analysis (figure 4b), the speciation rate for the time preceeding 30 Myr ago is significantly different from all other rate groups, thus clearly illustrating the initiation of the radiation at between 20 and 30 Myr ago. The local clocks (figure 4c) show a much more complex pattern, and between 2 and 4 Myr ago there was a significant increase in the speciation rate.

4. DISCUSSION

(a) *Progression of radiation*

The molecular clock hypothesis is rejected, reflecting the high among-lineage rate heterogeneity. We obtained different results using three methods for adjusting the rates among the lineages. NPRS resulted in the basal long branches (figure 1) being much shortened, leading to an early estimated date for the initiation of the *Restio*-clade radiation (*ca.* 42 Myr ago), in the Middle Eocene. Consequently, the time to speciation is initially short (3.07 ± 2.95 Myr), and then becomes long, peaking at the

Late Miocene value of 8.04 ± 6.81 Myr. As a result, the speciation rate between 12 and 50 Myr ago was significantly higher than the speciation rate in the last 4 Myr. Global and local clocks both resulted in long basal branches, thus shifting the initiation of the *Restio*-clade radiation to *ca.* 30 Myr ago. For global clocks, the subsequent time to speciation varies from 3.01 ± 2.308 Myr to 1.24 ± 1.518 Myr. For local clocks, the time between speciation events fluctuates substantially from 5.45 ± 4.63 Myr to 1.01 ± 1.48 Myr, with the recent time intervals generally with a shorter time interval between speciation events.

For the rest of the discussions and analyses here we will use the NPRS results, although there seems as yet no convincing way to choose between these results. There is clearly a need for more theoretical work and better algorithms that could address these differences. The more careful analyses, such as penalized likelihood (Sanderson 2002), are more difficult to implement for such large datasets.

The results are mostly consistent with the notion that the radiation of the Cape Restionaceae has continued without abrupt changes for the past 20 Myr. There is no evidence of any abrupt interruptions in the lineage accumulation rate, as could be expected from extinction events, nor is there any indication of sudden increases in the speciation rate, as was demonstrated for *Ehrharta* by Verboom *et al.* (2003). However, this is taken over the whole family, and if smaller clades were analysed such sudden changes might become evident, determined by the ecological idiosyncracies of these smaller clades. For larger clades, a degree of homogenization in the speciation rate is to be expected, resulting in a smoother lineage-accumulation rate.

Nee *et al.* (1994) suggested that the relationship between the speciation and extinction rates in a lineage could be determined by the relationship between the effective speciation rate at the apex of the LTT plot, relative to the main portion of the curve. A high extinction rate is manifested by an apparent increase in speciation at the apex of the curve, the 'pull of the present', i.e. the presence of lineages resulting from recent speciation events and which are not yet extinct. Thus, according to Nee *et al.* (1994) the

Figure 3. Lineage time plots for the *Restio*-clade (see figure 1), plotted (a) for the species richness not log-transformed, and (b) for the species richness log-transformed. Different rate scaling options were used: (i) NPRS, as implemented in TREEEDIT; (ii) global clock, as implemented in PAUP*; and (iii) local clocks, as implemented in PAML. These are based on the tree selected in figure 1.

Table 2. Significance values for the changes in speciation rates between the 10 time periods (0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–14, 14–20, 20–30 and 30–50 Myr ago). (Lower left triangle, NPRS rate modification; upper right triangle, global clock assumption. Significant differences ($p = 0.05$) indicated with asterisks.)

	0	2	4	6	8	10	12	14	20	30
0		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
2	0.792		0.774	0.428	0.861	0.843	0.878	0.821	0.884	0.968
4	0.257	0.252		0.176	0.543	0.492	0.784	0.452	0.511	0.654
6	0.438	0.505	0.819		0.547	0.384	0.156	0.366	0.301	0.169
8	0.13	0.121	0.525	0.459		0.99	0.641	0.966	0.944	0.736
10	0.071	0.052	0.356	0.328	0.851		0.567	0.972	0.92	0.663
12	0.001*	0.000*	0.004*	0.007*	0.042	0.047		0.501	0.589	0.816
14	0.016*	0.007*	0.098	0.112	0.424	0.509	0.151		0.879	0.585
20	0.011*	0.005*	0.065	0.076	0.305	0.363	0.26	0.771		0.698
30	0.006*	0.004*	0.034*	0.039*	0.154	0.181	0.622	0.415	0.586	

shape of the Restionaceae tree would indicate a very low extinction rate.

These results are consistent with the nested radiations documented for *Pelargonium* (Geraniaceae) by Bakker *et al.* (2004). Taken all together they probably indicate a continuous increase in species richness for the genus over the past 18 Myr. However, the species sampling in *Pelargonium* is still too incomplete to make a complete analysis of the speciation rates. The only other group with an almost complete sampling is the Iridaceae genus *Moraea* (Goldblatt *et al.* 2002), and this also appears to suggest a continuous rate of increase in species richness. Possibly the pattern illustrated in Restionaceae might be a common pattern for the Cape clades.

(b) Dating the initiation of the Cape radiations

The range of dates for the origin of the Restionaceae radiation ranges from 20 to 42 Myr ago. This is older than any other radiations documented from the Cape flora (table 3).

There could be several problems with the initiation dates for the African Restionaceae. First, there could be problems with the molecular evolution rate adjustments to accommodate non-clock-like behaviour (Sanderson 2002). However, our estimates take into account a number of possible rate adjustments, and we expect that these estimates include the range of possible rates.

The second problem could be with the calibration of the Restionaceae chronogram. We primarily used a fossil record of Restionaceae from the earliest Tertiary, from Banke in southern Africa (Scholtz 1985). Restionaceae pollen contains some unusual morphological features, which makes the identification of some of their pollen types unambiguous (Linder & Ferguson 1985), and the Banke Restionaceae fossils contain the unique synapomorphies of Restionaceae. The dates of the Banke deposits are not likely to be inaccurate (Scholtz 1985), and are backed by a series of more recent African and Australian deposits (reviewed in Linder *et al.* 2003). Thus, if anything, our initiation estimates underestimate the age of the Restionaceae radiation,

Figure 4. Mean time to speciation, calculated for 2 Myr time intervals from 0 to 14 Myr ago, then from 14 to 20 Myr ago, then 20 to 30 Myr ago, and finally for 30+ Myr ago. The error bars are standard deviations. (a) Using NPRS as implemented in TREEEDIT for the rate correction, (b) using a global clock as implemented in PAUP*, and (c) using local clocks as implemented in PAML. These are based on the tree selected in figure 1.

because it is unlikely that these are fossils of the first African Restionaceae. However, calibration dates based on a monocot-wide analysis leads to younger age estimates of the start of the radiation by 30% (Wikström *et al.* 2001; Bremer 2002). Using these dates consequently results in a 30% adjustment in all age estimates.

Similarly, there could be problems with proposed ages of the other clades. The impact of using different rate correction methods in these other studies has not been investigated. Furthermore, calibration of chronograms remains problematic. There are few local fossils that allow internal calibration points, and the only analyses that have internal calibrations (e.g. from a portion of the study group, or connected to it with a very short branch length) are of *Phylica* (dated by the age of the volcanic South Atlantic and Indian Ocean islands) and Restionaceae (by fossil deposits in a volcanic lake). Importing rates from other lineages, as was done for *Heliophila* and *Ehrharta*, is dubious, because of the extensive rate variation across the Angiosperms. Using secondary dates (usually from Wikström *et al.* (2001)) may also be problematic, and the authors (Wikström *et al.* 2004) note that the terminal node ages are often large underestimates. They presume that this is a result of the severe undersampling of the terminal lineages. The younger estimates of Restionaceae fall within the range of the dates estimated from both Iridaceae and

Pelargonium using the Wikström dating. Thus, if these latter clades were more completely sampled, perhaps the ranges would be similar to those of Restionaceae.

An additional problem with incompletely sampled clades is that if the basal lineages of the clades were not included in the sample, then the start of the radiation can be missed. This is evident in Restionaceae, where neither *Restio ambiguus* nor *Platycaulos* were placed basally on morphological evidence. Not sampling either of these two here would have reduced the estimated age of the radiation by 20%. A similar error in *Phylica* could increase the initiation time by at least 20%, in other words to over 10 Myr ago.

The hypothesis that all to-date-investigated clades initiated their radiations at the same time would be difficult to reject statistically, because there are so many uncertainties in the establishment of the radiation dates. However, the substantial differences in the suggested dates, and the lack of overlap in the ranges in the estimated dates, makes it unlikely that they are contemporaneous. Although we did not calculate the error around our age estimates, the almost threefold difference in the age estimates between the *Phylica* radiation and that of Restionaceae is most probably bigger than the error estimates. The available data rather indicate that the initiation of the radiations were spread over a longer time period, with the earliest being Restionaceae (Late Oligocene) and the most recent being *Phylica* and *Ehrharta* in the Late Miocene. It is also possible that some of the other lineages, not yet investigated, could show earlier or even later dates for the initiation of this radiation.

The implication here is that there may not have been a single trigger for the radiation in the Cape flora. A popular candidate has been the initiation of modern, summer-dry climates in the Late Miocene, heralded by the start of the Benguela cold water upwelling on the Atlantic Coast of southern Africa (Siesser 1978; Siesser & Dingle 1980; Linder 2003). However, the accumulating molecular phylogenetic evidence points towards the possible role of multiple factors and events, and the impacts of each of these must be evaluated in light of the particular biology of particular lineages, as was done for Rushioideae by Klak *et al.* (2004).

(c) Summed diversity of the Cape flora

The initiation dates of the radiations of clades including some 1400 species from the CFR have been postulated (table 3). By using the initiation dates and the species richness of the clades in the CFR, and by assuming that their lineage accumulation patterns are similar to those of Restionaceae, the lineage diversity for each clade for any time period can be calculated. For these data the global increase in diversity through the Neogene was calculated by summing the projected species richness for each clade for 2 Myr intervals since the Oligocene (figure 5). From this it is evident that at 12 Myr ago these lineages, which currently include 1400 species (15% of the flora of the CFR or 30% of the species richness of the Cape clades) included only 315 species (100 species if we assume that the speciation rate stays constant, as was suggested by the global clock analysis). By 8 Myr ago this number had increased to 618 (200 with constant rates), by the beginning of the Pliocene to 800 (500 with constant rates), and the beginning of the Pleistocene to just over 1200 (800 with constant rates). Thus, 200 of the modern 1400 species (*ca.*

Table 3. Summary of other radiation dates determined to date. (PL, penalized likelihood.)

taxon	date (Myr ago)	calibration	reference
<i>Phyllica</i> (Rhamnaceae)	8–7	Atlantic, Indian Ocean islands	Richardson <i>et al.</i> (2001b)
<i>Pelargonium</i> (Geraniaceae)	22–18, secondary radiation 10	Family age from Wikström <i>et al.</i> (2001)	Bakker <i>et al.</i> (2004)
<i>Ehrharta</i> (Poaceae)	9.8–8.7	subfamily age from Bremer (2002)	Verboom <i>et al.</i> (2003)
Ixiodeae (Iridaceae)	ca. 20	Family age from Wikström <i>et al.</i> (2001)	Reeves <i>et al.</i> (2001); Goldblatt <i>et al.</i> (2002)
<i>Moraea</i> (Iridaceae)	ca. 15	Family age from Wikström <i>et al.</i> (2001)	Reeves <i>et al.</i> (2001); Goldblatt <i>et al.</i> (2002)
<i>Heliophila</i> (Brassicaceae)	10.4–7.8	rates from <i>Gossypium</i> and Brassicaceae	A. Mühlhausen and K. Mummenhoff, personal communication
<i>Indigofera</i> (Fabaceae)	13.08 ± 3.52 (PL); 17 ± 2.56 (NPRS); 6.04 ± 0.96	<i>MatK</i> dates for legume nodes Wojciechowski (2003)	Schrire <i>et al.</i> (2003)

Figure 5. Changes in diversity of the Cape clades during the Neogene. Solid line, number of species of the Cape clades investigated; dashed line, proportion of the area occupied by Cape flora clades, with the possible environmental triggers indicated.

15%) are of Pleistocene origin if we apply the Restionaceae NPRS species accumulation model. However, if we assume that the speciation rate stays constant (as was suggested by the global clock analysis), this changes to 600 of the modern 1400 species (*ca.* 40%) being of Pleistocene origin. The equivalent figures for the Pliocene–Pleistocene are 500 species (35%) with the NPRS model and 800 species (*ca.* 65%) for the constant accumulation model.

Thus, although the radiations in these lineages started between the Late Miocene and the Late Oligocene, much of their modern species richness dates at most from the Pliocene. This is consistent with the suggestion by Goldblatt (1997) that the present Cape flora could only be distinguished after the beginning of the Pliocene, less than 5 Myr ago. Although Levyns (1964) did not put a date to her estimations, she seemed to suggest a similarly recent origin. Cowling & Pressey (2001) also imply a post-Miocene date. Evidently there is a large disjunction between the initiation of the radiations and the development of the modern diversity. However, a more precise estimation of the changes in species richness in the Cape flora over the Neogene would require a better quantification of the species

accumulation pattern, as it is evident that the different models have a major impact.

(d) From clades to vegetation types

Linder *et al.* (1992) suggested that extensive areas of fynbos were established in the Late Miocene as a consequence of climate change obliterating the tropical vegetation. The modern Cape flora clades then radiated into this vacant habitat. Presumably these clades soon approached the ecological diversity of the modern clades, but with few species, and these would then have had a wide ecological amplitude. With the passage of time, the available habitat is divided ever more finely among the newly evolved species. Modern analogues would be Renosterveld, very widespread within the CFR, but dominated by a single species, *Elytropappus rhinocerotis* (Taylor 1978), or the beech forests of central Europe. This model is similar to what may have happened after mass extinctions (Rosenzweig 1995), or the formation of new islands, such as Hawaii (Funk & Wagner 1995; Baldwin & Sanderson 1998). Consequently, we label this the 'tabula rasa' model. It is conceivable that the rate of speciation would then be determined by lineage specific attributes, such as physiological or morphological

innovations (reflected as habitat or pollinator specializations, for example) that enhance speciation rate (e.g. Hodges & Arnold 1995; Johnson 1996). By this model, fynbos vegetation, and the modern Cape floral biome, has been in existence possibly since the Miocene, maybe even earlier, as a major component of the vegetation of the CFR, but that its diversity is recent. Assuming that the current Cape lineages were all present by the late Miocene, which appears to be the case, a central prediction of this model is that the initiation of the radiation of the Cape floral clades should be simultaneous, in response to the extinction event, and be no older than the Late Miocene. This does not appear to be the case. Instead, the evidence to date is favouring a scenario in which the modern fynbos is comprised of clades with radiation dates staggered from 7 to perhaps 42 Myr ago. A second argument against this 'tabula rasa' model is the absence of extensive morphological diversity within the ecologically diverse lineages, as is seen in the other 'tabula rasa' radiations (e.g. Baldwin 1997).

The currently available evidence favours an alternative model, where adaptations to drought and fire gave a selective edge to the Cape floral clades over the tropical flora, and as summer drought increased during the Miocene, as indicated by fluctuations in strength of the Benguela upwelling, the area occupied by fynbos increased. This could have led to the competitive displacement and eventual exclusion of *Palmae* (Arecaceae), *Casuarinaceae* and *Winteraceae*, which were present in the CFR during the Miocene (Coetzee 1983, 1986; Coetzee & Pragłowski 1988), resulting in the fynbos vegetation, possibly with its attendant fire regime, occupying an ever increasing proportion of the area of the CFR (figure 5). In addition, geomorphological changes might also have made more habitat available into which the Cape clades could expand. For example, the limestone habitats of the coastal plains date from the Pliocene (Siesser & Dingle 1980). Furthermore, it is not clear what the effects of erosion during the Neogene might have been on the Cape mountains, and possibly more suitable oligotrophic habitat might have become available there (Linder 2003). Central to this model is that contrary to the 'tabula rasa' model, fynbos did not invade into a vacant area, but competitively displaced the existing vegetation. This model is consistent with the small number of radiation clades (few that had the necessary adaptations), the lack of morphological diversity within the radiating clades, and the different times of the initiation of the radiations. Although it seems clear that the modern Cape floral elements were not all in place at once, the paucity of palaeobotanical data prevents an understanding of how gradual the previous mesic floral elements were displaced or went extinct.

This gradual model is also consistent with the available lineage-level reconstructions. Bakker *et al.* (2004) found evidence that the radiation in *Pelargonium* started in the east, in a more even-rainfall environment, and that the most recent radiation was within the summer-dry west coast area. Similarly, an early study on the Restionaceae genus *Thamnochortus* (Linder & Mann 1998) indicated an evolutionary progression from all-year rainfall areas into summer-drought areas. This was corroborated by a more detailed ecological–evolutionary investigation of the genus (Linder & Hardy 2004) which showed that the ancestral

habitat was a higher-rainfall area, and that coastal and summer-dry habitats were derived within the genus. For the grass genus *Ehrharta*, Verboom *et al.* (2003) were able to demonstrate a radiation of the genus out of the mesic mountains onto the presumably newly seasonally arid coastal plains in the Late Miocene. These detailed studies corroborate Margaret Levyn's insightful statement from 1964, 'these unwieldy genera are the direct result of intensive speciation in the west.' (Levyns 1964, p. 102).

The vegetation changes described for the Australian Neogene by Kershaw *et al.* (1994) would also fit this gradual model. With gradual climatic change during the Neogene, the mosaic of vegetation types changes such that, at a regional scale, the proportional representation of the different vegetation types changes.

An investigation of the ancient Cape lineages that did not radiate (Levyns 1962; Linder 2003) shows that they are largely restricted to areas with a year-round supply of water, and occur in fire-protected habitats. Typical examples of such lineages would be *Prionium*, *Metrosideros*, *Platylophus*, *Cunonia*, *Brabejum* and *Hyenanche* (Linder *et al.* 1992). It is tempting to see them as relicts of a once more widespread mesic flora in the CFR, a flora of which possibly *Winteraceae* and *Casuarinaceae* might also have been part. The gradual deterioration of climatic conditions favourable to this mesic vegetation type, along with the attendant fire regimes, would have led to a gradual and perhaps staggered competitive displacement or exclusion of the mesic floral elements by the modern Cape lineages, leading to their respective radiations (Linder *et al.* 1992).

5. CONCLUDING REMARKS

We are making progress in simultaneously clarifying or corroborating aspects of the Levyns–Goldblatt hypothesis, that the modern species richness of the Cape flora is the result of radiation into the aridifying habitats of the western portion of the CFR. This process may have started in the Late Oligocene, and continued through the Late Miocene into the Pliocene, with a series of nested lineage-specific radiation initiation dates.

We are now working towards synthesizing the results of several studies to determine the overall patterns in the flora, thus avoiding the inevitable clade-based idiosyncracies of ecophysiological requirements and historical accidents. More exciting, we can start exploring the relationships between taxic diversification and the formation of vegetation types, and so progress towards understanding the evolution of biomes. However, for this process to continue, we need more data from ecologically divergent clades and, in particular, data from the huge genus *Erica* and the ecologically very well-known *Proteaceae* would be invaluable. But most important would be more detailed palaeobotanical information: this could be the only reliable source of information on the shifting balance between vegetation types. Palaeobotanical data would also be an immensely valuable source of data against which to test our post-dictions based on extant taxa.

This paper is part of ongoing research on the evolution of the Cape flora, funded by the Swiss Science Foundation, the Claraz Schenkung, the SANW, and the National Geographic Society. Philip Moline is thanked for providing sequences of *Askidiosperma*, *Chondropetalum*, *Dovea* and *Elegia*. Numerous

people have been involved in many discussions about the evolution of the Cape flora, and have contributed to the ideas presented here. We thank all of them.

REFERENCES

- Bakker, F. T., Culham, A., Marais, E. & Gibby, M. 2004 Nested radiation in Cape *Pelargonium*. In *Plant species-level systematics: new perspectives on pattern and process* (ed. F. T. Bakker, L. W. Chatrou, B. Gravendeel & P. B. Pelter), Königstein: Koeltz. (In the press.)
- Baldwin, B. G. 1997 Adaptive radiation of the Hawaiian silversword alliance: congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. In *Molecular evolution and adaptive radiation* (ed. T. J. Givnish & K. J. Systma), pp. 103–128. Cambridge University Press.
- Baldwin, B. G. & Sanderson, M. J. 1998 Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proc. Natl Acad. Sci. USA* **95**, 9402–9406.
- Bolus, H. 1886 *Sketch of the flora of South Africa*. Cape Town: Richards and Sons.
- Bremer, K. 2002 Gondwanan evolution of the grass alliance of families (Poales). *Evolution* **56**, 1374–1387.
- Briggs, B. G., Marchant, A. D., Gilmore, S. & Porter, C. L. 2000 A molecular phylogeny of Restionaceae and allies. In *Systematics and evolution of monocots*, vol. 1 of *Proc. 2nd Int. Conf. on the Comparative Biology of the Monocots*, Sydney, September 1998 (ed. K. L. Wilson & D. A. Morrison), pp. 661–671. Melbourne: CSIRO.
- Chase, M. W. & Albert, V. A. 1998 A perspective on the contribution of plastid *rbcL* DNA sequences to angiosperm phylogenetics. In *Molecular systematics of plants II: DNA sequencing* (ed. D. E. Soltis, P. S. Soltis & J. J. Doyle), pp. 488–507. Boston, MA: Kluwer.
- Chiang, T. & Schaaf, B. A. 2000 Molecular evolution of the *atpB-rbcL* noncoding spacer of chloroplast DNA in the moss family Hylocomiaceae. *Bot. Bull. Acad. Sinica* **41**, 85–92.
- Coetzee, J. A. 1983 Intimations on the Tertiary vegetation of southern Africa. *Bothalia* **14**, 345–354.
- Coetzee, J. A. 1986 Microfloral elements in the Neogene of the southwestern Cape Province and their phytogeographical significance. *Palaeoecol. Afr.* **17**, 21–30.
- Coetzee, J. A. & Pragowski, J. 1988 Winteraceae pollen from the Miocene of the southwestern Cape (South Africa). *Grana* **27**, 27–37.
- Cowling, R. M. & Pressey, R. L. 2001 Rapid plant diversification: planning for an evolutionary future. *Proc. Natl Acad. Sci. USA* **98**, 5452–5457.
- Cuénoud, P., Martinez, M. A. D., Loizeau, P. A., Spichiger, R., Andrews, S. & Manen, J. F. 2000 Molecular phylogeny and biogeography of the genus *Ilex* L. (Aquifoliaceae). *Ann. Bot.* **85**, 111–122.
- Eldenäs, P. & Linder, H. P. 2000 Congruence and complementarity of morphological and *trnL-F* sequence, and the phylogeny of the African Restionaceae. *Syst. Bot.* **25**, 692–707.
- Felsenstein, J. 1981 Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **35**, 292–303.
- Felsenstein, J. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Funk, V. A. & Wagner, W. L. 1995 Biogeographic patterns in the Hawaiian Islands. In *Hawaiian biogeography. Evolution on a hot spot archipelago* (ed. W. L. Wagner & V. A. Funk), pp. 379–419. Washington, DC: Smithsonian Institution Press.
- Goldblatt, P. 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Ann. Missouri Bot. Gard.* **65**, 369–436.
- Goldblatt, P. 1997 Floristic diversity in the Cape Flora of South Africa. *Biodivers. Conserv.* **6**, 359–377.
- Goldblatt, P. & Manning, J. 2000 *Cape plants. A conspectus of the Cape flora of South Africa*. Pretoria, South Africa: National Botanical Institute.
- Goldblatt, P. & Manning, J. C. 2002 Plant diversity of the Cape Region of southern Africa. *Ann. Missouri Bot. Gard.* **89**, 281–302.
- Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J. C., Barraclough, T. G. & Chase, M. W. 2002 Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Mol. Phylogenet. Evol.* **25**, 341–360.
- Goloboff, P. A. 1993 *NONA*. New York: Published privately.
- Good, R. 1974 *The geography of the flowering plants*, 4th edn. London: Longman.
- Hilu, K. W. & Liang, H. P. 1997 The *matK* gene: sequence variation and application in plant systematics. *Am. J. Bot.* **84**, 830–839.
- Hodges, S. A. & Arnold, M. L. 1995 Spurring plant diversification: are floral nectar spurs a key innovation? *Proc. R. Soc. Lond. B* **262**, 343–348.
- Johnson, S. D. 1996 Pollination, adaptation and speciation models in the Cape flora of South Africa. *Taxon* **45**, 59–66.
- Kershaw, A. P., Martin, H. A. & McEwen Mason, J. R. C. 1994 The Neogene: a period of transition. In *History of the Australian vegetation* (ed. R. S. Hill), pp. 299–327. Cambridge University Press.
- Klak, C., Reeves, G. & Hedderson, T. A. 2004 Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature* **427**, 63–65.
- Levyns, M. R. 1962 Possible Antarctic elements in the South African flora. *S. Afr. J. Sci.* **58**, 237–241.
- Levyns, M. R. 1964 Presidential address, migrations and origin of the Cape flora. *Trans. R. Soc. S. Afr.* **37**, 85–107.
- Linder, H. P. 1984 A phylogenetic classification of the genera of the African Restionaceae. *Bothalia* **15**, 11–76.
- Linder, H. P. 2001 *The African Restionaceae*. Cape Town: Contributions from the Bolus Herbarium.
- Linder, H. P. 2003 The radiation of the Cape flora, southern Africa. *Biol. Rev.* **78**, 597–638.
- Linder, H. P. & Ferguson, I. K. 1985 On the pollen morphology and phylogeny of the Restionales and Poales. *Grana* **24**, 65–76.
- Linder, H. P. & Hardy, C. R. 2004 Species richness in the Cape flora: a macroevolutionary and macroecological perspective. In *Plant species-level systematics: new perspectives on pattern and process* (ed. F. T. Bakker, L. W. Chatrou, B. Gravendeel & P. B. Pelter). Königstein: Koeltz. (In the press.)
- Linder, H. P. & Mann, D. M. 1998 The phylogeny and biogeography of *Thamnochortus* (Restionaceae). *Bot. J. Linn. Soc.* **128**, 319–357.
- Linder, H. P., Meadows, M. E. & Cowling, R. M. 1992 History of the Cape flora. In *The ecology of fynbos: nutrients, fire and diversity* (ed. R. M. Cowling), pp. 113–134. Oxford University Press.
- Linder, H. P., Eldenäs, P. & Briggs, B. G. 2003 Contrasting patterns of radiation in African and Australian Restionaceae. *Evolution* **57**, 2688–2702.
- Manen, J., Natali, A. & Ehrendorfer, F. 1994 Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergene region. *Pl. Syst. Evol.* **190**, 195–211.

- Marloth, R. 1908 Das Kapland, insonderheit das Reich der Kapflora, das Waldgebiet und die Karroo, pflanzengeographisch dargestellt. In *Wissenschaftliche Ergebnisse der deutschen Tiefsee-Expedition auf dem Dampfer 'Valdivia' 1898-1899* (ed. C. Chun), pp. 1–427. Jena, Germany: Gustav Fischer.
- Nee, S., Mooers, A. O. & Harvey, M. 1992 Tempo and mode of evolution revealed from molecular phylogenies. *Proc. Natl Acad. Sci. USA* **89**, 8322–8326.
- Nee, S., Holmes, E. C., May, R. M. & Harvey, P. H. 1994 Extinction rates can be estimated from molecular phylogenies. *Phil. Trans. R. Soc. Lond. B* **344**, 77–82.
- Nixon, K. C. 1999 The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* **15**, 407–414.
- Nixon, K. C. 2002 *WINCLADA*. Ithaca, NY: published by the author.
- Posada, D. & Crandall, K. A. 1998 MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Rambaut, A. & Charleston, M. 2004 *TREEEDIT: an application for organising, viewing and manipulating sets of phylogenetic trees*. Oxford: published privately.
- Reeves, G., Chase, M. W., Goldblatt, P., Rudall, P. J., Fay, M. F., Cox, A. V., Lejeune, B. & Souza-Chies, T. 2001 Molecular systematics of Iridaceae: evidence from four plastid DNA regions. *Am. J. Bot.* **88**, 2074–2087.
- Richardson, J. E., Pennington, R. T., Pennington, T. D. & Hollingsworth, P. M. 2001a Rapid diversification of a species-rich genus of neotropical rainforest trees. *Science* **293**, 2242–2245.
- Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C. B., Linder, H. P., Reeves, G. & Chase, M. W. 2001b Rapid and recent origin of species richness in the Cape flora of South Africa. *Nature* **412**, 181–183.
- Rosenzweig, M. L. 1995 *Species diversity in space and time*. Cambridge University Press.
- Sanderson, M. J. 1997 A non-parametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* **14**, 1218–1231.
- Sanderson, M. J. 2002 Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* **19**, 101–109.
- Scholtz, A. 1985 The palynology of the upper lacustrine sediments of the Arnot Pipe, Banke, Namaqualand. *Ann. S. Afr. Mus.* **95**, 1–109.
- Schrire, B. D., Lavin, M., Barker, N. P. & Cortes-Burns, H. 2003 Towards a phylogeny of *Indigofera* (Leguminosae-Papilionoideae): identification of major clades and relative ages. In *Advances in legume systematics, part 10. Higher level systematics* (ed. B. B. Klitgaard & A. Bruneau), pp. 269–302. Kew, UK: Royal Botanic Gardens.
- Siesser, W. G. 1978 Aridification of the Namib Desert: evidence from ocean cores. In *Antarctic glacial history and world palaeoenvironments* (ed. E. M. Van Zinderen Bakker), pp. 105–113. Rotterdam, The Netherlands: A. A. Balkema.
- Siesser, W. G. 1980 Late Miocene origin of the Benguela upwelling system of northern Namibia. *Science* **208**, 283–285.
- Siesser, W. G. & Dingle, R. V. 1980 Tertiary sea-level movements around southern Africa. *J. Geol.* **89**, 83–96.
- Swofford, D. L. 2002 *PAUP*: phylogenetic analysis using parsimony (* and other methods)*, v. 4. Sunderland, MA: Sinauer.
- Taberlet, P., Gielly, L., Patou, G. & Bouvet, J. 1991 Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Mol. Biol.* **17**, 1105–1109.
- Takhtajan, A. 1986 *Floristic regions of the world*. Berkeley, CA: University of California Press.
- Taylor, H. C. 1978 Capensis. In *Biogeography and ecology of southern Africa* (ed. M. J. A. Werger), pp. 171–229. The Hague: Junk.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997 The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.
- Turner, G. T. 1999 Explosive speciation of African cichlid fishes. In *Evolution of biological diversity* (ed. A. E. Magurran & R. M. May), pp. 113–129. Oxford University Press.
- Van Wyk, A. E. & Smith, G. F. 2001 *Regions of floristic endemism in southern Africa*. Hatfield, UK: Umdaus Press.
- Verboom, G. A., Linder, H. P. & Stock, W. D. 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.
- Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. 2003 Origin of the superclade of cichlid fishes from Lake Victoria, East Africa. *Science* **300**, 325–329.
- White, F. 1983 *The vegetation of Africa*. Paris: UNESCO.
- Wikström, N., Savolainen, V. & Chase, M. W. 2001 Evolution of the angiosperms: calibrating the family tree. *Proc. R. Soc. Lond. B* **268**, 2211–2220. (doi:10.1098/rspb.2001.1782)
- Wikström, N., Savolainen, V. & Chase, M. W. 2004 Angiosperm divergence times: congruence and incongruence between fossils and sequence divergence estimates. In *Telling the evolutionary time* (ed. P. C. J. Donoghue & M. P. Smith), pp. 142–165. Boca Raton, FL: CRC Press.
- Wojciechowski, M. F. 2003 Reconstructing the phylogeny of legumes: an early 21st century perspective. In *Advances in legume systematics, part 10. Higher level systematics* (ed. B. B. Klitgaard & A. Bruneau), pp. 5–35. Kew, UK: Royal Botanic Gardens.
- Yang, Z. 1997 PAML: a program package for phylogenetic analysis by maximum likelihood. *CABIOS Computer Application. Biosci.* **13**, 555–556.
- Yoder, A. D. & Yang, Z. H. 2000 Estimation of primate speciation dates using local molecular clocks. *Mol. Biol. Evol.* **17**, 1081–1090.

GLOSSARY

- CFR: Cape Floristic Region
 LTT: lineage-through-time
 MPT: most parsimonious trees
 NPRS: non-parametric rate smoothing
 TBR: tree bisection–reconnection