

Frequent and parallel habitat transitions as driver of unbounded radiations in the Cape flora

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The enormous species richness in the Cape Floristic Region (CFR) of Southern Africa is the result of numerous radiations, but the temporal progression and possible mechanisms of these radiations are still poorly understood. Here, we explore the macroevolutionary dynamics of the Restionaceae, which include 340 species that are found in all vegetation types in the Cape flora and are ecologically dominant in fynbos. Using an almost complete (i.e., 98%) species-level time calibrated phylogeny and models of diversification dynamics, we show that species diversification is constant through the Cenozoic, with no evidence of an acceleration with the onset of the modern winter-wet climate, or a recent density-dependent slowdown. Contrary to expectation, species inhabiting the oldest (montane) and most extensive (drylands) habitats did not undergo higher diversification rates than species in the younger (lowlands) and more restricted (wetland) habitats. We show that the rate of habitat transitions is more closely related to the speciation rate than to time, and that more than a quarter of all speciation events are associated with habitat transitions. This suggests that the unbounded Restionaceae diversification resulted from numerous, parallel, habitat shifts, rather than persistence in a habitat stimulating speciation. We speculate that this could be one of the mechanisms resulting in the hyperdiverse Cape flora.

KEY WORDS: Cape Floristic Region, density-dependent radiation, diversification, habitat transitions, Restionaceae, unbounded radiation.

The Cape flora, with about 10,000 species in 90,000 km² (Manning and Goldblatt 2012) is remarkably species rich. Approximately half of this species richness is found in 33 clades (Linder 2003), many of which are largely restricted to the Cape Floristic Region (CFR), suggesting that this enormous species richness is the result of a limited number of radiations. This richness may be the result of accelerated diversification (Schnitzler et al. 2011, Valente and Vargas 2013), either when the lineages entered the region (e.g., Proteaceae [Sauquet et al. 2009], Rhamnaceae [Onstein et al. 2015], and *Erica* [Pirie et al. 2016]), or when they shifted from forest to the pyrophytic healthy “fynbos” vegetation (e.g., Penaeaceae [Onstein et al. 2014]). The accelerated diversification may have been the result, inter alia, of the physical and climatic heterogeneity of the region, frequent fires, or the highly specialized pollination syndromes (Goldblatt 1978; Johnson 2010; Schnitzler et al. 2011; Valente et al. 2012). However,

the temporal progression and underlying mechanisms of these radiations remain enigmatic.

The temporal progression of radiations is still poorly understood, partially because the investigation requires (almost) completely or randomly sampled phylogenies (Cusimano and Renner 2010). Theory predicts that adaptive radiations should be initiated when a lineage enters a new adaptive zone (Simpson 1944) or ecological opportunity (Schluter 2000), and should slow down as the available habitats are filled (Phillimore and Price 2008; Rabosky and Lovette 2008; Rabosky 2009a, b; Rabosky and Glor 2010; Rabosky and Hurlbert 2015). The mid- to late-Miocene establishment of summer drought (Dupont et al. 2011) and regular fire (Bytebier et al. 2010) have been postulated to provide the ecological opportunity that may have triggered the Cape radiations (Goldblatt 1978; Linder et al. 1992; Onstein et al. 2014; Bengtson et al. 2015). Density-dependent slow-downs, the second

prediction, are elegantly illustrated by the radiation of anolids on the Caribbean islands (Rabosky and Glor 2010), but have been demonstrated in only few Cape radiations (e.g., Hoffmann et al. 2015; Slingsby et al. 2014). Furthermore, ecological and spatial patterns may also complicate the predicted temporal progression of radiations. For instance, biotic interactions and geographical isolation events due to habitat fragmentation, followed by dispersals, could lead to increasing diversification rates through time (Herrera 2017), leaving a signal of unbounded diversification dynamics. The Cape mountains are older than the coastal plains and probably date to the Palaeogene (Scharf et al. 2013), whereas the coastal plains may have been restructured by mid-Miocene and Pliocene uplift episodes (Partridge and Maud 2000; Cowling et al. 2009) and eustatic sea-level fluctuations. The greatest species richness in the Cape flora is found in the western mountains and the lowest on the southern coastal plains (Manning and Goldblatt 2012). This fits theoretical predictions, namely that the greatest diversity should be found in older environments (the “time-for-speciation” effect; Stephens and Wiens 2003), and in mountainous regions with a greater diversity of habitats and fragmented distribution ranges (Hoorn et al. 2013). The “time-for-speciation” hypothesis predicts unbounded accumulation of species. Furthermore, we expect that **larger contiguous habitats** (in the Cape, dryland habitats) should host more diversification than habitats fragmented into smaller patches (wetland habitats in the Cape) (Kisel and Barraclough 2010; Losos and Schluter 2000). Finally, the phylogenetic niche conservatism hypothesis predicts that when a lineage has occupied a habitat it should diversify within it, and habitat transitions should be rare events (Wiens et al. 2010). Consequently, habitat shifts should not be able to drive diversification.

Restionaceae constitute one of the most characteristic components of the Cape flora. The 340 species constitute the monophyletic subfamily Restionoideae (Briggs and Linder 2009), here referred to as “restios.” Although restios are found in all vegetation types in the Cape flora, they are ecologically dominant in most formations of fynbos, which is the most widespread and species-rich of these vegetation types (Rebello et al. 2006). Fynbos is a heathland occurring mostly on sandstone derived soils and is analogous to Californian chaparral and Mediterranean Maquis (Manning and Goldblatt 2012). Detailed ecological information has been compiled for all species (Linder 2001): they inhabit all fynbos habitats, from coastal plains to high mountain peaks and from wetlands to drylands. The first fossil (palaeopalynological) record of restios is from the early Palaeocene Banke deposits (Scholtz 1985), and they have subsequently been recorded in all southwestern African fossil plant deposits (Linder et al. 2003). It seems most probable that they have been part of the evolving fynbos vegetation and Cape flora throughout the Cenozoic. This suggests that they might be suitable for developing a “straw man”

diversification dynamics hypothesis for the Cape flora, although it should be noted that they cannot be taken as a surrogate for other Cape groups such as Orchidaceae, Aizoaceae, Proteaceae, or legumes.

Here, we first test whether the diversification of restios accelerated with the establishment of modern climates in the middle Miocene (the putative ecological opportunity), and whether there is any density-dependent slowdown in diversification rates, which would illustrate the adaptive radiation model. Second, we test whether (1) the greater diversity in the mountains is due to older occupation rather than a higher diversification rate, and (2) dryland habitats have a higher diversification rate than wetlands, as predicted if areal extent of the fragments is important. Finally, we evaluate which evolutionary model best describes habitat evolution and whether habitat transitions have driven diversification.

Materials and Methods

CHRONOGRAM

A chronogram of the restios was inferred from published plastid sequence data (Hardy et al. 2008) augmented by sequences of *atpB-rbcL* (266 species downloaded/52 species sequenced/data for 320 species), *trnK-matK* (262/47/309), *trnL-F* (277/52/329), *psbA-trnH* (0/156/156), and *rpl32-trnL* (0/49/49). In total, 356 new sequences have been deposited in GenBank (KX886361–KX886717; see details in Table S1). Thus, we sampled 335 restio species (representing 98% of all species) and two outgroups from Australasian Restionaceae, *Baloskion tetraphyllum* and *Sporodanthus tasmanicus* (see details in Table S1), each represented by a single specimen. Total DNA was isolated from silica-gel-dried culms using the DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, CA). DNA sequences were generated following the protocol of Hardy and Linder (2005) for *atpB-rbcL*, *trnK-matK*, and *trnL-F*. For *psbA-trnH* and *rpl32-trnL*, we used protocols for PCR amplification of Tate and Simpson (2003), and for sequencing those of Shaw et al. (2007). The concatenated matrix included ~30% missing data (7% for *trnK-matK*, 5% for *atpB-rbcL*, 1% for *trnL-F*, 53% for *psbA-trnK*, and 85% for *rpl32-trnL*; Table S1). Even though the amount of missing data seemed large, Zheng and Wiens (2015) have shown that missing data only have minor impacts on the accuracy of divergence-time estimates using BEAST.

Sequences were aligned using MUSCLE 3.6 (Edgar 2004), checked manually, and ambiguous fragments excluded. The combined matrix included 10,121 characters. The substitution model used for Bayesian inferences and molecular dating analyses was determined using hierarchical likelihood ratio tests as implemented in MODELTEST 3.06 (Posada and Crandall 1998). The optimal model for all five plastid regions was GTR + I + G (Yang 1994). The concatenated DNA matrix was analyzed in MrBayes 3.2.6 (Huelsenbeck and Ronquist

2001); four parallel Markov chain Monte Carlo (MCMC) were run for 5,000,000 generations with trees sampled every 1000 generations. Two independent analyses were performed to check whether convergence on the same posterior distribution was reached. To assess the adequacy of sampling from the posterior probability distribution and to determine the burn-in, we plotted changes in likelihood over 2,000,000 generations using the *sump* function and compared them across the two analyses. The consensus tree from the MrBayes analysis was used as a starting tree for the subsequent molecular dating analysis.

Dating was performed using a Bayesian analysis assuming a relaxed clock, with uncorrelated rates sampled from a lognormal distribution, implemented in BEAST 1.8 (Drummond et al. 2006, 2013; Drummond and Rambaut 2007). Although there are numerous Restionaceae fossil pollen deposits from the Cape, these can be confidently placed only to subfamily, thus we use the earliest to constrain a minimum age for the stem group of the (African) restios. However, there is a much larger set of calibration points available in the Poales (Table S2). It has been shown that to improve age estimates and avoid age underestimation, it is advised to (1) calibrate nodes within the study group (i.e., reducing distances from calibration nodes), and (2) avoid undersampling-related groups (i.e., increasing tree imbalance) (Shaul and Graur 2002; Linder et al. 2005; Duchêne et al. 2015). Thus, we used a two-step dating procedure. We first estimated the age of the restios from a large set of calibration points within the sparsely sampled Poales. We then used this secondary calibration to date the almost completely sampled species-level phylogeny of the restios. This approach avoided the tree imbalance that would have resulted from the comprehensive sampling of restios and very sparse sampling of the rest of Poales, but still allowed us to use the much richer fossil set of the Poales. In the first step, we estimated the crown age of Restionaceae from a dating analysis of the Poales by downloading from GenBank *rbcL*, *ndhF*, and *matK* sequences for 44 Poales species representing all the major families within the order, and three outgroup species belonging to the sister Arecales (see Bouchenak-Khelladi et al. 2014, and details in Table S3). We selected Poales species to bracket calibration nodes, based on the fossil record (Table S2). We unlinked substitution rates between the three chloroplast regions. The minimum ages were based on nine fossils (Table S2), and the maximum age set to 125 million years, as no monocot fossils have been described from the early Cretaceous (Gandolfo et al. 2002). Uniform priors best reflect the available fossil evidence, as we had a single sample per calibration point, and so could not justify the more complex shapes of other priors. In the second step, we calibrated the almost completely sampled species-level tree of restios. The 95% highest posterior densities (95% HPDs) of the crown node of Restionaceae, inferred during the first step (Fig. S1), was used as a secondary calibration for the stem node of restios, using a

normal distribution with the inferred median age as the mean and the 95% HPD as the SD (mean: 69.0 with SD: 5.5). In addition, the crown node of the *Thamnochortus spicigerus*–*T. pluristachyus* clade was calibrated with a uniform distribution (minimum = 1.0 and maximum = 5.0) because this clade is restricted to limestone habitats in Bredasdorp area (Linder and Mann 1998), which were exposed ~3 Ma by Pliocene tectonic uplift (Linder 2003; Rogers 1987). We unlinked substitution rates between the five plastid regions. For both analyses, we performed three runs of 100,000,000 chains, sampling every 1000 generations. We checked for convergence (i.e., ESS threshold > 700) and viewed statistics of each run using TRACER 1.5 (Rambaut et al., 2014), and, after removing 50,000,000 burn-in samples, we summarized the results using a maximum clade credibility (MCC) tree. Depending on the complexity of the analyses, we used either 100 or 1000 trees randomly sampled from the posterior distribution of the BEAST analyses for subsequent analyses.

TEMPORAL PROGRESSION OF THE RADIATION

We tested whether a single diversification regime is sufficient to account for diversification rates in the restios (e.g., whether these is rate variation among lineages) with BAMM 2.5.0 (Rabosky 2014), after excluding the outgroups, using the MCC tree. BAMM uses reversible-jump MCMC to select between models that vary in the number of diversification regimes, thus accounting for rate variation through time and among lineages. We ran two MCMCs for 100,000,000 generations with a sampling frequency of 1000 for each clade. We checked for convergence for each run by plotting the log-likelihood trace of the MCMC output file and checked that the effective sample sizes of the runs exceeded 200. Using the BAMMtools 2.1.5 package (Rabosky et al. 2014) in R, we identified the 95% credible set of distinct shift configurations and the overall best set of rate shifts given the data using Bayes factors.

We found no significant support for interclade diversification rate variation, so used the R package DDD 3.3 (Etienne et al. 2012) to test for density-dependent diversification slowdown over all restios. We compared the constant birth–death model, which assumes constant rates of speciation and extinction through time, against the density-dependent logistic (i.e., linear rate changes in diversification) and the density-dependent exponential (i.e., exponentially declining speciation rates as a function of extant lineage diversity at any point in time) models. We used the correct Akaike Information Criterion (AICcs) to identify the best-fit model.

All analyses were conducted on the MCC tree from the BEAST analysis and run in R 3.2.4 (R Development Core Team 2016). To incorporate phylogenetic uncertainty in the diversification analyses, we ran BAMM and DDD analyses on a set of 100 trees. We then computed the best shift configuration and the AICc for each tree in BAMM and DDD, respectively. Finally, to test the diversification patterns found in BAMM and DDD, we repeated

the analyses using RPANDA 1.2 (Morlon et al. 2016) and TESS 2.1.0 (Höhna et al. 2016) (see Methods S1 for details).

ECOLOGICAL AND SPATIAL PATTERNS IN THE RADIATION

To test the ecological and spatial prediction that the diversifications were faster in montane dryland rather than coastal plains wetland habitats, we scored all species as montane versus coastal plains, and wetland (impeded drainage) versus dryland (freely draining) habitats, using Linder (2001). Such a simplification is justified, as restios are largely restricted to oligotrophic or well-leached, usually sandy, soils, and are only found in full sun, and in winter-wet climates. Thus, the major ecological differences among the species are in soil drainage and altitude. The datasets are available on the DRYAD digital repository (<https://doi.org/10.5061/dryad.pc8r1>). Because both traits are binary, they were combined into four categorical habitats: (1) montane wetlands (MW), (2) montane drylands (MD), (3) coastal plains wetlands (CW), and (4) coastal plains drylands (CD), resulting in 184 MD, 63 MW, 60 CD and 14 CW species (see details in Table S4). The 16 missing species were assumed to be equally distributed among the habitat categories, but were not excluded in the diversification analyses.

We asked whether the diversification dynamics of four habitat categories (MW, MD, CW, CD) differed by integrating both temporal (i.e., older montane) and spatial (i.e., areally restricted wetlands) patterns. We used MuSSE (multistate speciation extinction), which extends BiSSE to multistate characters (FitzJohn 2012). We made likelihood functions with “make.musse” implemented in DIVERSITREE 0.9-3 (FitzJohn 2012). The 16 missing species were assumed to be equally distributed among the four habitat categories. We compared four MuSSE models: namely (1) an unconstrained model in which transition rate (q), extinction rate (μ), and speciation rate (λ) were allowed to vary; (2) a constrained model in which λ was kept constant; (3) one in which μ was kept constant; and (iv) one in which λ and μ were kept constant. Also, to test whether the best model was selected with enough power, we randomly distributed habitat categories (i.e., same frequency distribution and number of states as our observed data) over the tips of the phylogeny 100 times. For each of these 100 randomly distributed traits, we fitted the best model found and compared the observed and simulated AICs. To account for phylogenetic uncertainty, we calculated the maximum likelihood (ML) parameter estimates of the best model for each of 100 trees. The joint posterior sample was used to calculate the mean of each parameter and the 95% credibility intervals.

To test whether simplifying habitat categories into binary characters (i.e., montane vs. coastal plains and drylands vs. wetlands) would generate conflicting results, we ran BiSSE and HiSSE models (Maddison et al. 2007; Fitzjohn 2012; Beaulieu

and O’Meara 2016) (see details in Methods S2). Finally, to check whether diversification rates were associated with habitat transitions, we ran a full ClaSSE model, which implements cladogenetic character change and state-dependent diversification (Goldberg and Igić 2012) (see details in Methods S2).

The diversification patterns in the four habitats cannot be visually compared with an ordinary lineage-through-time plot, as internal nodes with ambiguous optimizations will be counted twice. Consequently, we estimated the MW, CW, MD, and CD lineage diversity at each internal node while accounting for the ML probabilities for node optimizations (Mahler et al. 2010). This method follows a three-step procedure: (1) estimating the ancestral states of each node in the tree using ML reconstruction, (2) estimating the number of lineages present in each habitat at the time of occurrence of that node by summing marginal probabilities, and (3) combining information about the number of extant lineages present in each with the estimate of the ancestral state of that particular node (Mahler et al. 2010). We determined whether an equal rate (ER) or an all rates different (ARD) model of habitat evolution is better, by calculating the fit of the habitats over 1000 trees, using the “BayesMultistate” method as implemented in BAYESTRAITS 2.0 (Pagel 1994; Pagel and Meade 2006; Venditti et al. 2011). We compared the likelihoods of the models with a likelihood ratio statistic, which is nominally distributed as a χ^2 with degrees of freedom equal to the differences in the number of parameters between the models. Having established the best model, we used the function “estDiversity” in the R Package PHYTOOLS 0.5-38 (Revell 2012), which implements the method of Mahler et al. (2010). Lineage density was computed as the sum of the posterior probabilities of ancestral state reconstruction times the summed marginal ancestral reconstructions across coextant edges (Revell 2012).

HABITAT SHIFTS AND DIVERSIFICATION

To determine the evolutionary pattern of the four broad habitat types, we used the function “fitDiscrete,” contained in the R package GEIGER 2.0.6 (Harmon et al. 2008). The function uses ML to estimate the best fit of the observed data to a topology under different evolutionary models. We tested the fit of the data over 1000 trees under six models: (1) “None,” where habitat transitions are constant through time; (2) “ λ ,” where trait changes reflect their shared evolutionary history (as expected under a Brownian motion) (Pagel 1999); (3) “ κ ,” where character transitions are associated with branching events, and are not influenced by branch length, when κ is close to 0 (Pagel 1999); (4) “ δ ,” where transitions are concentrated early or late in the tree when $\delta < 1$ or $\delta > 1$, respectively (Pagel 1999); (5) “EB,” where transition rates increase or decrease exponentially through time (Harmon et al. 2010), and (6) “White,” where rates do not follow a phylogenetic model. We used the AICc to select the optimal

This is a really strange way to add uncertainty in your data. It isn't biologically realistic to randomly assign states.... These aren't even a way to compare these because they are different data and a different model. We are grabbing 100 trees and putting the same model (HiSSE with everything free) on these different trees

They would've been much better if they gave the same estimates under the same model and compare them rather than this

Table 1. Model comparison of diversification models of African restios using Bayes factor in BAMM (Rabosky 2014), and AICc for DDD (Etienne et al. 2012), with the best models in bold.

Diversification models			
BAMM (Rabosky 2014)		Bayes factor	
No rate shift		0	
1 rate shift		2.87	
2 rate shifts		9.38	
3 rate shifts		29.16	
4 rate shifts		93.30	
DDD (Etienne et al. 2012)	LogL	AICc	ΔAICc
Constant birth–death	−1068.85	2141.74	0
Diversity-dependent linear	−1069.74	2145.55	3.81 (0.15–3.70)
Diversity-dependent exponential	−1083.19	2172.45	26.90 (12.11–26.23)

LogL indicates the log likelihood of the models. Values in parentheses indicate the AICc differences for 100 posterior trees.

model. We computed the transition probabilities between the four habitat categories inferred under the κ model of evolution (which was found to be optimal; see Results). Therefore, we used parsimony as implemented in MESQUITE 3.3 (Maddison and Maddison 2017), as parsimony does not take branch length into account. We estimated the number of habitat transitions by mapping the habitats over a randomly selected set of 1000 trees.

Results

CHRONOGRAM

The MCC phylogeny and the set of the 1000 posterior trees from the BEAST analyses are available on the DRYAD digital repository (<https://doi.org/10.5061/dryad.pc8r1>). The MCC with Bayesian posterior probabilities and 95% confidence intervals at the nodes is shown in Figure S2. The stem age of Restionoideae was estimated at 66.6 Ma (95% HPD: 56.9–78.4). The two major clades, Restieae and Willdenowieae, are both well supported (1.0 Posterior Probability; Fig. S2) and originated 42 Ma (95% HPD: 35.3–56.4) and 32.1 Ma (95% HPD: 26.6–44.3), respectively.

TEMPORAL PROGRESSION OF THE RADIATION

The hypothesis of a constant diversification for the restios was not rejected by any of the analyses we performed (Tables 1 and S5). The set of shifts configuration from BAMM 2.5.0 indicated no rate shift based on the MCC (Fig. S4), and for the set of 100 trees, we found no shift for 79, and one rate shift for 21, trees (Figs. 1A and S4; Table 1). This single shift may occur at one of two locations (Fig. 1A). The lineage through time plot showed a pattern of unbounded species proliferation (Fig. 1B). The absolute fit of the restio phylogeny to the constant rate birth–death model using posterior predictive simulation is shown in

Figure S3. The observed species number, lineage through time plot, and the gamma statistic indicated that the phylogeny fitted the constant rate model (Fig. S3). All four analyses converged to the same result (TESS analysis indicated that a constant birth–death model was best, whereas RPANDA found a pure birth constant speciation model [Table S5]) and DDD a constant birth–death (Table 1). Thus, all the analyses supported the absence of any density-dependent slowdown in diversification rate.

ECOLOGICAL AND SPATIAL PATTERNS IN THE RADIATION

We found no significantly higher diversification rates in any of the four habitat categories using the MuSSE speciation and extinction model (Table 2; Fig. 2A). The simulated AICc are higher than the observed (Fig. 2B), indicating that the best MuSSE model was selected with enough power and we can reject a Type 2 error. The inferred habitat transition rates from the MuSSE model indicate no significant differences because of overlaps of their 95% credibility intervals (Fig. S5). The lineage diversity first accumulated within montane drylands, then montane wetlands, while coastal habitats started accumulating diversity only ~15 Ma (Fig. 2C). However, there seemed to be a slowdown in lineage accumulation for coastal wetlands ~5 Ma (Fig. 2C).

For both montane/coast and dryland/wetland state-dependent diversification analyses, the CID-2 (i.e., character independent on dtwo states) null HiSSE models were the best (Table 3). Lineages in drylands or montane habitats were not associated with higher diversification rates because diversification process parameters accounted for trait-dependence of an unobserved hidden trait (Table 3), suggesting that diversification rate changes are not associated with habitat type or habitat shifts as configured here.

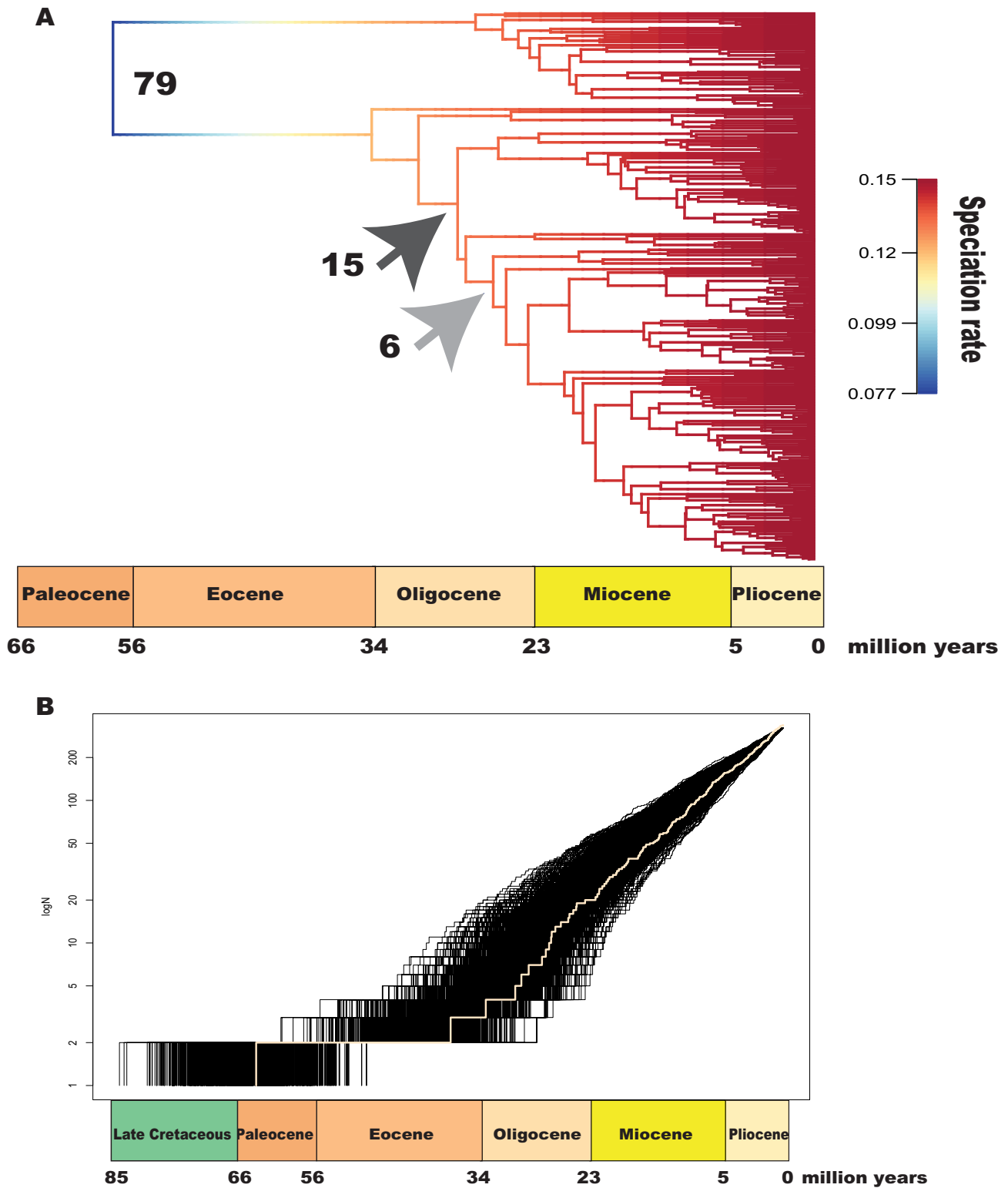


Figure 1. Diversification pattern in the African Restionaceae from the BAMM analyses (Rabosky 2014). (A) Speciation rates are shown along each branch of the phylogeny. Each color section of a branch represents the mean of the posterior density of speciation rate. Seventy-nine, 15, and six indicate the number of trees (from the 100 posterior trees tested) exhibiting no rate shift and one rate shift, respectively, after computing the best shift configuration. (B) Lineage through time plot of the African Restionaceae using 1000 trees. The white thick line indicates the MCC tree.

Table 2. Likelihood ratio test between MuSSE models where (1) speciation (λ) and extinction (μ) rates of montane wetlands (0), coastal wetlands (1), montane drylands (2), and coastal drylands (3) habitats are different; (2) λ and μ are equal; (3) λ are equal and μ are different; and (4) λ are different and μ are equal.

Models	k	$\ln(L)$	AIC	AICc	$\Delta AICc$
$\lambda_0 \neq \lambda_1 \neq \lambda_2 \neq \lambda_3, \mu_0 \neq \mu_1 \neq \mu_2 \neq \mu_3$	20	−1340.7	2721.3	2724.1	0
$\lambda_0 = \lambda_1 = \lambda_2 = \lambda_3, \mu_0 \neq \mu_1 \neq \mu_2 \neq \mu_3$	17	−1340.7	2715.4	2724.1	0
$\lambda_0 = \lambda_1 = \lambda_2 = \lambda_3, \mu_0 = \mu_1 = \mu_2 = \mu_3$	14	−1349.8	2727.6	2742.3	18.2
$\lambda_0 \neq \lambda_1 \neq \lambda_2 \neq \lambda_3, \mu_0 = \mu_1 = \mu_2 = \mu_3$	17	−1345.1	2724.1	2732.9	8.8

df is degrees of freedom and $\ln(L)$ indicates the log likelihoods of the models. The best model is indicated in bold.

HABITAT SHIFTS AND DIVERSIFICATION

The best evolutionary model for habitat preferences was the κ model (Table 4), showing that transitions are related to speciation events (Fig. 3A; κ being not significantly different from 0; Table 4), rather than to branch lengths. This suggest that the rate of habitat transitions is related to the number of cladogenetic events rather than to time (branch length), but species being in a new habitat did not undergo changes in their diversification rates (Fig. 2A).

The ancestral habitat in the Cape was inferred to be montane (Fig. 2C). The first occupation of coastal habitats occurred sometimes between the Oligocene and the early Miocene (95% HPD: 18.4–30.8 Ma) (Fig. 2C). Our optimizations, using the ARD model ($df = 1, P < 0.0001$), are ambiguous as to whether the ancestral habitat was wet- or drylands (Figs. 2C and S6), consequently, these could not be dated. Parsimony mapping shows that there are 93 transitions on a phylogeny with 335 nodes, thus habitat changes are optimized to 28% of all the internodes on the tree; 69% of all habitat changes are either from montane drylands to montane wetlands, or from montane drylands to coastal dryland habitats (Table 5), revealing massive parallel habitat changes.

When considering transition probabilities under the κ model with 1000 posterior trees, the highest probability involves CW to MW, then MW to MD, CD to MD, and MD to CD (Fig. 3B).

Discussion

CHRONOGRAM

The chronogram is largely congruent with previously published phylogenies of the restios (Eldenäs and Linder 2000; Hardy et al. 2008; Litsios et al. 2014), revealing two clades, the Restieae and Willdenowieae (Fig. S2). Our molecular dating indicates that restios were present in southern Africa throughout the Cenozoic, and the lineage through time plot suggests that they were species poor during the Palaeogene, and became diverse during the Neogene (Fig. 1B), with most genera differentiating during the Oligocene and Miocene, and most species during the Plio-Pleistocene. This finding is consistent with the fossil pollen

record, which indicates a continuous presence of restios in the Cape throughout the Cenozoic, but with most records from the Miocene–Pliocene (Linder et al. 2003).

TEMPORAL PROGRESSION OF THE RADIATION

We find no support for any shifts in diversification in the restios (Figs. 1A and B, S3, and S4; Tables 1 and S5), suggesting a constant proliferation of species because of the Palaeocene. Thus, the adaptive radiation model is rejected for restios, and our results are more consistent with the “time-for-speciation” model (Stephens and Wiens 2003). Both the hypotheses of a climate- or fire-driven mid-Miocene trigger to the radiation, and the hypothesis of a density-dependent slowdown, have been rejected for the restios.

For many Cape clades, early to middle Miocene crown ages have been reported. These Miocene origins could reflect the time when (1) the clade entered the fynbos flora (e.g., Penaeaceae; Onstein et al. 2014), or (2) the lineage dispersed into southern Africa, for example, Apiaceae (Calvino et al. 2016). It could also be the result of the phylogenetic level of clade that was selected for the analysis, for example, *Moraea* (Iridaceae) (Goldblatt et al. 2002). In a study including *Babiana* and *Moraea* (Iridaceae), *Protea* (Proteaceae) and Podalyrieae (Fabaceae), which entered the Cape flora between the Eocene and the Late Miocene, Schnitzler et al. (2011) also could not demonstrate diversification rate changes. Consequently, there is no consistent signal of rate acceleration in the Cape flora, either in the Miocene or at any other time, thus no general response to any environmental changes. There is, therefore, no unambiguous indication of specific ecological opportunity for the Cape flora. Possibly there are several (such as oligotrophic soils, winter rainfall, and fire on a decadal scale) that might have been established asynchronously during the Eocene to Miocene.

We show that diversification in the restios is unbounded, thus not density dependent, and with no evidence of habitat saturation, except for coastal wetlands (Fig. 2C). The occurrence of density-dependent slowdowns in diversification is still generally disputed (Rabosky and Lovette 2008; Rabosky 2009a, b; Rabosky and Glor 2010; Wiens 2011; Harmon and Harrison 2015; Rabosky and

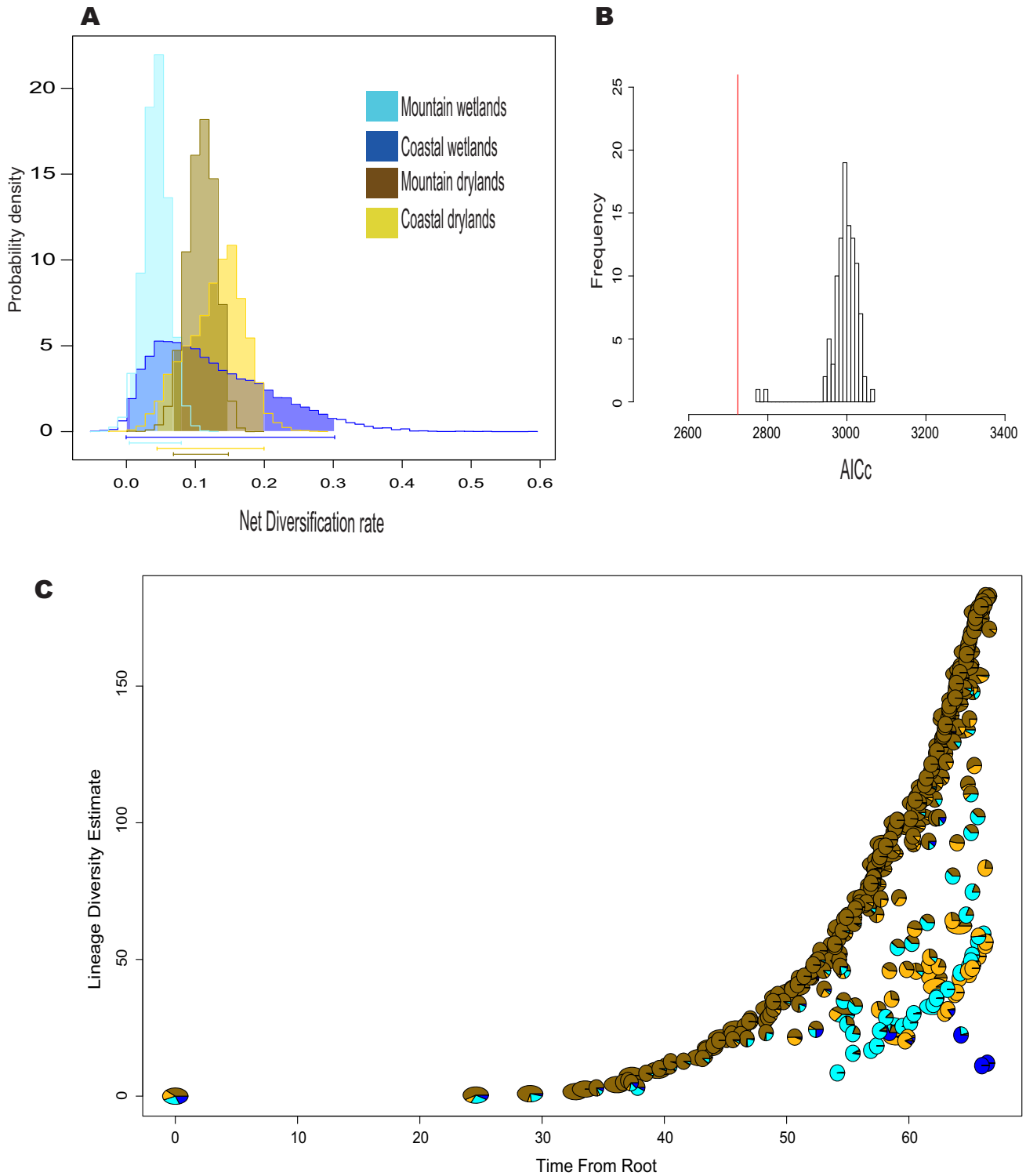


Figure 2. (A) MuSSE analysis of the four habitat categories. (B) Histogram of the simulated AICc using 100 randomized datasets using the best MuSSE model selected for our observed data. The red line indicates the observed AICc values, showing that the observed AICc is significantly lower than the simulated ones. (C) The lineage diversity accumulation through time in the four habitat categories of restios. Each point reflects a node on the MCC tree from the BEAST analysis. The diversity estimate is the marginal probabilities of the four habitats multiplied by the summed probabilities of each habitat category of all ancestral nodes. The colors indicate the four habitats as for panel A.

Table 3. Results from fitting lineage diversification models.

Models	k	ln(L)	AIC	AICc	ΔAICc	wtAICc
BiSSE: $\lambda_0 \neq \lambda_1, \mu_0 \neq \mu_1$	6	-1203.3	2418.6	2418.86	25.97	< 0.0001
BiSSE: $\lambda_0 \neq \lambda_1, \mu_0 = \mu_1$	5	-1203.3	2416.6	2416.79	23.89	< 0.0001
BiSSE: $\lambda_0 = \lambda_1, \mu_0 = \mu_1$	4	-1207.8	2423.5	2423.72	30.83	< 0.0001
BiSSE: $\lambda_0 = \lambda_1, \mu_0 \neq \mu_1$	5	-1207	2423.9	2424.19	31.29	< 0.0001
HiSSE: CID-2	12	-1183.95	2391.9	2392.89	0	1
HiSSE: CID-2 <i>q</i> 's equal	5	-1201.53	2413.05	2413.24	20.35	< 0.0001
HiSSE: CID-4	11	-1192.98	2407.96	2408.8	15.9	< 0.0001
HiSSE: CID-4 <i>q</i> 's equal	9	-1197.79	2413.57	2414.14	21.25	< 0.0001
ClaSSE: full model	10	-1196.98	2414.02	2415.12	22.23	< 0.0001
BiSSE: $\lambda_2 \neq \lambda_3, \mu_2 \neq \mu_3$	6	-1194.9	2401.9	2402.06	17.51	< 0.0001
BiSSE: $\lambda_2 \neq \lambda_3, \mu_2 = \mu_3$	5	-1195.2	2400.5	2400.59	16.04	< 0.0001
BiSSE: $\lambda_2 = \lambda_3, \mu_2 = \mu_3$	4	-1198.5	2405	2405.12	20.57	< 0.0001
BiSSE: $\lambda_2 = \lambda_3, \mu_2 \neq \mu_3$	5	-1198.5	2407	2407.19	22.64	< 0.0001
HiSSE: CID-2	12	-1179.77	2383.54	2384.55	0	0.628
HiSSE: CID-2 <i>q</i> 's equal	5	-1187.94	2393.87	2386.06	1.51	0.295
HiSSE: CID-4	11	-1182.95	2387.9	2388.75	4.2	0.077
HiSSE: CID-4 <i>q</i> 's equal	9	-1199.11	2416.21	2416.79	32.24	< 0.0001
ClaSSE: full model	10	-1184.13	2388.31	2389.14	4.59	0.081

The models are compared with AICc scores and Akaike's weights (wtAICc). ΔAICc scores indicate the difference between the candidate models and the best-fitting one (in bold). Speciation (λ) and extinction (μ) rates of wetlands (0), drylands (1), montane (2), and coastal plains (3). k indicates the number of parameters. CID-2 and CID-4 refer to character-independent models assuming that the evolution of binary character is independent of the diversification process, where CID-2 and CID-4 account for trait-dependent diversification on two and four states of a hidden trait, respectively (Beaulieu and O'Meara 2016). The full ClaSSE model (Goldberg and Iqic 2012) determines whether diversification rates were associated with habitat transitions for one descendant species or for both. *q*'s equal indicates that transitions are kept equal.

Table 4. Model comparisons of evolutionary models for the four habitat categories (i.e., MW, MD, CW, and CD) using Bayes Factors.

Models	-(lnL)	AICc	Bayes factor	Significance
κ	304.84 (299.50-310.90)	636.86 (626.18-648.98)	0	0.13***
λ	311.66 (303.61-345.66)	650.49 (634.39-690.15)	13.64	
δ	318.88 (309.90-331.03)	664.42 (642.78-685.07)	28.08	
None	320.32 (311.26-331.93)	665.66 (647.52-688.86)	30.96	
EB	351.11 (339.65-359.54)	729.44 (710.32-752.65)	92.54	
White	351.13	708.34	92.58	

White = a nonphylogenetic model; EB = early-burst model (Harmon et al., 2010); None = a model of rate constancy through time; λ = a covariance model (Pagel, 1999); κ = a speciation model (Pagel, 1999); δ = a time-dependent model (Pagel, 1999).

One thousand posterior trees were used in the analysis. The mean of the log-likelihoods (lnL) and the AICc are reported and their distribution across the 1000 trees. The best model is the κ model shown in bold. The significance column indicates the *P* values for κ (not significantly different to 0) with *** indicating *P* < 0.001.

Hurlbert 2015). Unbounded radiations have been reported, inter alia, for Neotropical ovenbirds and honeycreepers, associated with the new habitats created by Andean uplift (Derryberry et al. 2011), for African catfishes associated with river-system evolution (Day et al. 2013) and with tropical African frogs (Liedtke et al. 2016), suggesting that continuous diversification is more likely in tropical continental areas, where habitat saturation is prevented by geomorphological or climatic change and the ecological complexity of the landscape. The same Andean-

uplift-driven South American system can, however, also result in density-dependent patterns (Pincheira-Donoso et al. 2015). Unbounded diversification can also be sustained by dispersal to new regions, as demonstrated for oceanic passerine birds (Fritz et al. 2012). However, the CFR does not fit any of these models. It is geographically limited, and often referred to as being "island like" (Linder 2003). It is thought to have been largely geomorphologically stable (Linder and Verboom 2015), and the radiations largely endemic. Furthermore, it is climatically temperate.

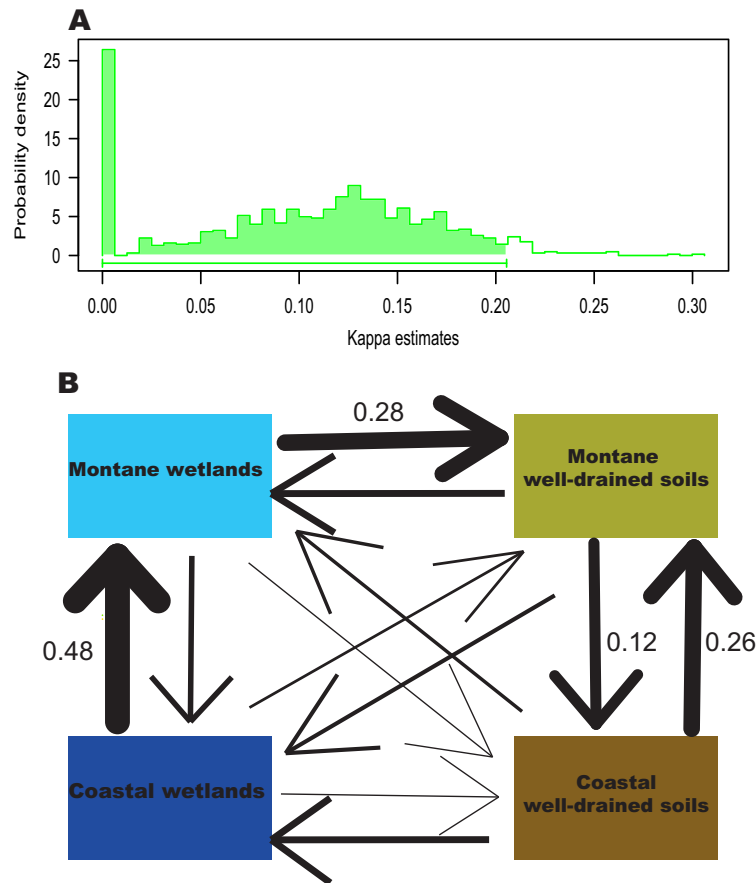


Figure 3. (A) The distribution of the kappa (κ) parameter (i.e., where character transitions are associated with branching events, and are not influenced by branch length, when κ is close to 0; Pagel, 1999) calculated over 1000 posterior trees. This shows that κ is very close to 0. (B) The transition probabilities between the four habitat categories inferred under the κ model of evolution with the width of the arrows being proportional to the mean probabilities.

It is still unclear whether the absence of a density-dependent diversification slowdown in the restios is unusual for the CFR. Density-dependent diversification signatures have been reported for *Tetraria* (Cyperaceae) (Slingsby et al. 2014), *Protea* (Proteaceae) (Valente et al. 2010; Hoffmann et al. 2015), *Stoebe* (Asteraceae), *Moraea* (Iridaceae), and *Satyrium* (Orchidaceae) (Hoffmann et al. 2015). However, Valente and Vargas (2013) showed that the richness of 19 Cape lineages scaled with age of the lineages, suggesting no density dependence. This is in stark contrast to lineages from the Mediterranean basin. Consistent with these results, Schnitzler et al. (2011) could not reject a continuous diversification model for *Moraea*, *Babiana*, Podalyrieae and *Protea* once they had corrected for undersampling, and Linder and Bouchenak-Khelladi (2015) showed a continuous diversification pattern for the danthonioid grasses.

MONTANE AND DRYLAND RADIATIONS

Based on our habitat optimizations, the first appearances of coastal lineages occurred between the Oligocene and the early Miocene

Table 5. The number of habitat changes in the African restios, as determined by a parsimony optimization over 1000 trees.

Habitat change	Minimum	Maximum	Average	Percentage
CW→CD	0	1	0.041	0.04450233
CW→MD	0	2	0.238	0.25833062
MW→CW	0	3	1.349	1.46423532
CW→MW	1	4	1.523	1.65309888
CD→MW	0	5	1.717	1.8636709
CD→CW	2	3	2.063	2.23922718
MW→CD	0	6	2.209	2.3976989
CD→MD	1	11	5.084	5.51828937
MW→MD	2	16	7.199	7.81395854
MD→CW	4	9	7.209	7.82481276
MD→MW	20	38	29.42	31.933138
MD→CD	27	40	34.078	36.9890372

MD = montane dry; MW = montane wet; CD = coastal dry; CW = coastal wet. The average is calculated over the full set of 1000 trees, and the percentage changes from these averaged changes.

(Figs. 2C and S6), which probably preceded the first Neogene uplift in the Cape (Partridge and Maud 2000). This geological event may have exposed part of the continental shelf, and also led to an accelerated erosion stripping off a laterite capping making underlying clay soils available (Cowling and Procheş 2005; Cowling et al. 2009). A second Pliocene uplift may have rejuvenated these processes. Although the Pliocene uplift was focused in the eastern side of Southern Africa, it raised the coastal limestones above sea level in the CFR. In addition, the onset of the winter rainfall conditions in the middle to late Miocene (Dupont et al. 2011) may have resulted in the expansion of dry to seasonally dry, open vegetation on the coastal plains, making these more suitable for fynbos (and so restios) by the middle Miocene (Hoffmann et al. 2015).

We found that habitats, whether considered separately or combined, are not associated with shifts in diversification rates (Tables 2 and 3; Fig. 2A). Older and spatially extensive habitats did not undergo faster diversification. This is inconsistent with the results of Schnitzler et al. (2011), who showed that the Cape mountains had a higher diversification rate than the lowlands or the Succulent Karoo. A more detailed analysis of the habitat effect might be required to critically test whether all habitats have the same diversification rate.

PARALLEL HABITAT SHIFTS AS A DRIVER OF DIVERSIFICATION

Habitat evolution in the restios showed two interesting attributes: the number of transitions is more related to the number of speciation events than branch length, and they are remarkably frequent. Furthermore, simple transitions (i.e., involving a change in either elevation or moisture) are much more frequent than complex transitions (i.e., involving changes in both elevation and moisture). Plotting habitat transitions on the phylogeny shows that clusters of closely related species share the same habitat (Fig. S6), but transitions are common, especially from montane dry habitats to montane wet and coastal dry habitats. Averaged over 1000 phylogenies, these add up to 64 transitions, or 69% of all transitions, indicative of numerous parallel habitat transitions.

This is consistent with the interpretation that frequent habitat transitions may play a role in the unbounded diversification in the restios, in addition to diversification within each habitat. Diversification within habitats may be the result of allopatric isolation following dispersal, as it has been shown for *Inga* (Mimosoideae) and other tree genera in the Amazonian rainforest (Dexter et al. 2017). Repeated transitions into new habitats may trigger minor radiations, defined as numerous and small (i.e., involving few species rather than a large clade). The fact that transition into novel habitats may trigger radiations is well known in angiosperms (Hughes and Eastwood 2006), fungi (Kraichak et al. 2015), reptiles (Losos and Mahler 2010), and Neotropical ro-

dents (Parada et al. 2015). This mechanism, applied to interbiome transitions, has been postulated to be an important mechanism for generating diversity in biodiversity hotspots (Linder and Verboom 2015). Here, we suggest it may also operate at the habitat level, at the very local scale of the CFR. We predict that each separate transition will be ecologically somewhat differentiated, thus not in direct competition with lineages in other transitions. These parallel habitat transitions are postulated to play a similar role as Andean uplift is thought to have played in the diversification of ovenbirds and woodcreepers in the Neotropics (Derryberry et al. 2011), or as drainage development in African catfish radiations (Day et al. 2013).

Our model makes a number of predictions for the Cape flora. We expect that (1) unbounded radiations will be the norm, (2) habitat transitions followed by small radiations will be typical, (3) at least some of these small radiations will be the result of allopatric speciation in the fragmented Cape mountains, and (4) these parallel transitions will be ecologically differentiated, thus not competing with each other. It seems probable that such a diversification requires long-term environmental stability in which the ecologically differentiated parallel transitions can occur. Such stability allows each radiation to become adapted and specialized, facilitating a parallel transition.

DATA ARCHIVAL

The doi of our data is <https://doi.org/10.5061/dryad.pc8r1>.

AUTHOR CONTRIBUTIONS

YBK and HPL designed the study. YBK performed statistical analyses and HPL collected data. YBK and HPL interpreted the results and wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1. Chronogram of the Poales molecular dating using *rbcl* and *ndhF*.

Fig. S2. Summarized chronogram of the African Restionaceae using plastid markers.

Fig. S3. Absolute fit of the African Restionaceae MCC phylogeny to the constant-rate birth–death model using posterior predictive simulation in TESS (Höhna et al. 2016).

Fig. S4. The set of rate shift configuration from BAMM 2.5.0 (Rabosky 2014) on the MCC tree, which indicates the absence of any rate shift with 93% confidence.

Fig. S5. 95% Posterior distribution of the transition rates inferred from the best MuSSE model from Coastal Wetlands, Montane Wetlands, Coastal Drylands and Montane Drylands, respectively.

Fig. S6. Ancestral habitat reconstructions under an ARD model with marginal probabilities at ancestral nodes calculated over 1000 posterior trees in BayesTraits (Pagel, 1994; Pagel et al., 2006; Venditti et al., 2011).

Table S1. Table of taxa, voucher information, accession numbers for GenBank (in bold are those taxa sequenced for this study).

Table S2. Fossils used to calibrate the Poales chronogram.

Table S3. Table of accession numbers from GenBank for the Poales dating.

Table S4. Habitat coding for African restios.

Table S5. Model comparison of diversification models of African restios using Bayes Factor in TESS (Höhna et al. 2016), and AICc for RPANDA (Morlon et al. 2016), with the best models in bold.