Research article

Diversification rate shifts in the Cape Floristic Region: The right traits in the right place at the right time

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ABSTRACT

Species diversity patterns are the product of diversification rate variation, but the factors influencing changes in diversification rates are poorly known. Radiation is thought to be the result of ecological opportunity: the right traits in the right environment at the right time. We test this in the Cape Floristic Region (CFR) of South Africa, in which pyrophytic heathland (fynbos) and non-pyrophytic Afromontane forest occur interdigitated. We infer transitions from forest to fynbos in three Cape clades (Penaeaceae, Phyliceae and Diosmeae) and test if they are associated with diversification rate shifts and the evolution of functional traits linked to fire, high insolation and seasonal drought. We estimate diversification rate shifts using maximum likelihood and use phylogenetic comparative methods to show that forest to fynbos shifts were associated with decreases in leaf area and specific leaf area and preceded or coincided with increases in diversification rates. Furthermore, we show that Penaeaceae, Phyliceae and Diosmeae species are typical members of their vegetation types in terms of their traits. The diversification rate shifts of Penaeaceae and Phyliceae are dated to the Miocene, when postulated aridification-driven changes in the CFR fire regimes may have triggered expansion of the fynbos at the cost of forest, providing an ecological opportunity for the diversification of fynbos lineages.

Introduction

The Cape Floristic Region (CFR) (Goldblatt, 1978) of South Africa, with its unusually elevated angiosperm species diversity of ca. 9000 species in an area of 90,000 km² and 68.8% endemism at the species level (Manning and Goldblatt, 2013), has been the arena for some remarkable radiations (Linder, 2003, 2005, 2008; Verboom et al., 2009). The CFR has a predominantly Mediterranean-type climate, characterized by wet winters and dry summers (Kottek et al., 2006). The species-rich, pyrophytic, nutrient-poor heathland called 'fynbos' dominates the region and co-occurs with species-poor, non-pyrophytic evergreen Afromontane forest, which is restricted to isolated fire-sheltered enclaves, often along rivers (Mucina and Geldenhuys, 2006; Rebelo et al., 2006). The high species richness and endemism of fynbos may be the result of a high *in situ* diversification rate.

Theory predicts that diversification rates may be increased by 'ecological opportunity' (Baldwin and Sanderson, 1998; Moore and Donoghue, 2007), in combination with the appropriate traits (Arakaki et al., 2011; Drummond et al., 2012; Near et al., 2012). Fynbos may constitute the critical ecological opportunity in the CFR. Fire, low soil nutrients, summer-drought and high insolation are typical for the fynbos vegetation (Keeley et al., 2012). These conditions limit the range of viable ecological strategies in a community, creating communities filtered for functionally similar species (Webb et al., 2002; Verdú and Pausas, 2007). These conditions therefore generate a 'functional trait syndrome' by selecting for or filtering out those species that lack these traits (Ackerly, 2004a), and the evolution of functional traits associated with fynbos vegetation is therefore expected to have influenced the evolutionary fate of the clades entering this system. This 'fynbos syndrome' includes highly branched slender twigs with a high density of narrow leaves with low leaf moisture content, and a shrubby growth-form, all typical for sclerophyllous, fire-spreading vegetation (van Wilgen et al., 1990; Schwilk and Ackerly, 2001; Ackerly, 2004b; Belcher et al., 2010). These lead to leaves with a high tissue density and low surface area per unit mass (low Specific Leaf Area, SLA). Decreases in leaf area and SLA have been shown to be related to increasing drought and nutrient limitation (Fonseca et al.,

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2000; Ordoñez et al., 2009), and small leaves are correlated with high insolation (Wright et al., 2004; Cornwell and Ackerly, 2009). Species in arid or seasonally-arid regions have been shown to have a low SLA (Fonseca et al., 2000; Ackerly, 2004a, but see Wright et al., 2004), and evolution of evergreen sclerophyllous shrubs in fire-prone Mediterranean ecosystems in general is convergent (Mooney and Dunn, 1970).

Here we use three typical Cape flora clades (Linder, 2003) – Penaeaceae Sweet ex. Guill. (Myrtales), Phyliceae Reissek ex Endl. (Rhamnaceae, Rosales) and Diosmeae DC. (Rutaceae, Sapindales) – to test the hypothesis that the evolution of low SLA and small leaves is correlated with the entry into fynbos and was followed by increased rates of diversification. Penaeaceae, Phyliceae and Diosmeae are the only three Cape clades to our knowledge that are almost entirely restricted to the CFR and are represented both in the evergreen Afromontane forest and the fynbos. Furthermore, these clades are well studied phylogenetically (Richardson et al., 2001, 2004; Rutschmann, 2006; Rutschmann et al., 2007; Trinder-Smith et al., 2007), and sequence data were therefore readily available. We first asked whether there were any diversification rate shifts in the three clades (**Q1**). To this end, we inferred time-calibrated phylogenetic trees and used a likelihood method to determine diversification rate shifts while correcting for incomplete taxon sampling. Then we asked whether vegetation type and leaf traits show correlated evolution (**Q2a**). We tested the null hypothesis that vegetation type and leaf trait shifts are not concordant, indicating that the evolution of low SLA and small leaves did not coincide with the transition to fynbos. We used phylogenetic comparative methods to infer ancestral states and to test for correlated evolution between vegetation type and traits. Furthermore, we tested whether forest and fynbos Penaeaceae, Phyliceae and Diosmeae are typical members of their respective vegetation types in terms of their leaf traits (**Q2b**), by comparing community trait profiles in paired forest-fynbos plots in the CFR. Finally, where we found positive answers to Q1 and Q2, we asked whether the greater diversity in fynbos compared to forest was due to an elevated diversification rate in fynbos and the evolution low SLA and small leaves (**Q3**), in other words, whether vegetation type shifts, trait shifts and diversification rate shifts were concordant between clades and in time.

Overall, we show that shifts in vegetation type and leaf traits have either preceded or coincided with the accelerated *in situ* diversification of fynbos taxa, the syndrome of low SLA and small leaves therefore being a 'precursor' for accelerated diversification. We discuss these results in the context of the complex diversification history of the CFR.

Materials and methods

Phylogenetic data

We assembled an alignment of 5123 base pairs (bp) for 25 of the 33 species of Penaeaceae and eight outgroup species of Alzataceae and Crypteroniaceae from previously published chloroplast (*rbc*L, *ndh*F and *rpl*16-intron) and nuclear (ribosomal 18S and 26S) DNA sequence data (TreeBASE study number S1802) (Schönenberger and Conti, 2003; Rutschmann et al., 2007). The Phyliceae dataset consisted of previously published *trn*L-F and internal transcribed spacer (ITS) sequence data (Richardson et al., 2001) which we expanded with additional sequence data (GenBank accession numbers are provided in Table S1). DNA extraction, DNA sequencing and sequence aligning procedures were performed as described in Richardson et al. (2001). The final dataset included 47 of the 136 species of Phyliceae augmented with 10 outgroup species from other Rhamnaceae genera in the ziziphoid group, and the alignment consisted of 1847 bp. The aligned Diosmeae dataset

consisted of 2522 bp of previously published plastid sequence data (*trn*H-*psb*A intergenic spacer, *atp*B-*rbc*L intergenic spacer and *rpl*16-intron) (TreeBASE study number S1814) (Trinder-Smith et al., 2007). All currently recognized genera of Diosmeae, each represented by between one and five species, were included in this study, resulting in a total of 26 of the 276 species of Diosmeae, and four outgroup species of genera in Rutaceae.

Timing of divergences

The joint posterior distribution of topologies and divergence times were estimated for all three clades using Bayesian MCMC implemented in BEAST 1.7.1 (Drummond and Rambaut, 2007) under a lognormal relaxed clock model and a pure-birth speciation process. The GTR + Γ nucleotide substitution model was used for all loci, and genomic regions were unlinked to accommodate differences in mean substitution rates between chloroplast and nuclear DNA. Priors for the mean substitution rates and the calibrated Yule (Heled and Drummond, 2011) were estimated in a test run in which the prior of the calibrated node was constrained for its mean age. A final run was performed using the estimated substitution rates of the test run as prior distributions for these rates, and relaxing the calibration prior to allow for uncertainty in the timing of divergences. We explored the use of normal and lognormal calibration prior distributions in Penaeaceae, Phyliceae and Diosmeae, but as age estimates did not differ much between both approaches, we will only present the results based on the normally distributed priors here (for BEAST settings and comparison of divergence time estimates under normal and lognormal priors see Table S2). We performed two MCMC runs of 20 (Penaeaceae, Diosmeae) or 30 (Phyliceae) million generations, sampling every 1000 generations. The first 10% generations were discarded as burn-in. Convergence of the model parameters of the MCMC chains was checked in Tracer 1.5 (Rambaut and Drummond, 2007), and topological convergence using the online service AWTY (Nylander et al., 2008).

Rutschmann et al. (2007) calibrated the Penaeaceae and related families using six fossils and several calibration sets. They estimated the crown age of the clade containing Crypteroniaceae, Alzataceae and Penaeaceae to be between 72.8–81.5 Ma. We set the prior distribution of the age of this group as a normal distribution with a mean of 77.15 and a standard deviation of 2.7 Ma, and constrained it to be monophyletic. Speciation was set to a calibrated Yule prior with a lognormal distribution with a log (mean) of 0.11 and log (standard deviation) of 0.27.

Richardson et al. (2004) calibrated the Rhamnaceae using the mean stem age estimates of Wikström et al. (2001) of 62 and 64 Ma, resulting in an age estimate of 22.9–23.6 ± 3.1 Ma for Phyliceae. We followed this calibration, and set a normal distribution with a mean of 23.3 and a standard deviation of 2.1 Ma. The calibrated Yule prior containing the monophyletic Phyliceae was set with a lognormal distribution with log (mean) of 0.056 and a log (standard deviation) of 0.38. We acknowledge that a tertiary calibration may not be reliable for estimating divergence times. Although fossils within the Phyliceae are lacking, there are fossils associated with the genera *Ceanothus* and *Colubrina*, which we included as outgroup lineages. We tested the reliability of the tertiary calibration by performing an analysis in which we calibrated the stem nodes of *Ceanothus* (uniform prior, 18–96) and *Colubrina* (uniform prior, 28.4–96) based on, respectively, a fossil of *Ceanothus precuneatus* from Middlegate (USA) (Axelrod, 1985) and a fossil of *Colubrina spireaefolia* from Florrisant (USA) (Manchester, 2001) and comparing the obtained highest posterior density (HPD) of the Phyliceae crown node age of this analysis to the HPD obtained from the analysis using a tertiary calibration. The minimum age of the calibration prior was determined by the age of the fossils; the maximum age