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Abstract

Dense branching and spines are common features of plant species in ecosystems with high mammalian herbivory pressure. While dense branching and spines can inhibit herbivory independently, when combined, they form a powerful defensive cage architecture. However, how cage architecture evolved under mammalian pressure has remained unexplored.

Here we show how dense branching and spines emerged during the age of mammalian radiation in the Combretaceae family, and diversified in herbivore-driven ecosystems in the tropics.

Phylogenetic comparative methods revealed that modern plant architectural strategies defending against large mammals evolved via a stepwise process. First, dense branching emerged under intermediate herbivory pressure, followed by the acquisition of spines that supported higher speciation rates under high herbivory pressure.

Our study highlights the adaptive value of dense branching as part of a herbivore defence strategy, and identifies large mammal herbivory as a major selective force shaping the whole plant architecture of woody plants.

Main text

INTRODUCTION

Disturbances exert strong ecological filtering on plant communities[1,2]. The pioneering work by Warming and Vahl[3], and Raunkiaer's[4] growth form classifications, laid the foundation for understanding how disturbance shapes the evolution of whole plant architectures. Frost[4,5], drought[6,7,8] and fire[9,10,11] are among the most widely recognised disturbances driving plant architectures, and have triggered the evolution of structural and functional traits such as: (i) prostrate architectures, facilitating frost avoidance[12] (ii) modularity, increasing drought tolerance[13]; and (iii) underground storage organs and resprouting capacity, allowing regeneration after fire[14,15,16,17]. By contrast, the effects of mammalian herbivory on plant architectural evolution and plant diversification have barely been investigated[18], despite a rapidly expanding literature on the macroecological effects of vertebrate herbivores on vegetation.

Evolutionary radiations can occur when acquired traits encounter novel environmental conditions. These traits, hereafter designated as key confluences, are defined by Donoghue and Sanderson[19] as a combination of intrinsic trait innovations with extrinsic ecological opportunities (e.g an environmental change) whose interaction increases diversification rates. Key confluences have been proposed between plant architectural trait innovations and climate shifts[12,20], but the effects of varying herbivory regimes on evolutionary processes in plants have not been tested. Nonetheless, it is widely apparent that large mammal herbivores alter vegetation structure through their feeding pressure, filtering species communities and organising biome distribution in Africa[21,22,23], South America[24,25] and Australia[26,27,28]. Large mammal herbivore species richness and diversity is greatest in semi-arid, open vegetation (grasslands, savannas and shrublands)[29], suggesting that those biomes experience high herbivory pressure and offer the greatest potential for key confluences between plant traits and mammalian herbivory to arise[30]. Wetter monsoonal climates typically support high fire frequencies (mesic savannas[31,32,33]), yet can still sustain high herbivore densities[29,34], while rainforests are likely much less impacted by vertebrate herbivores, notwithstanding the effects of elephants where they occur[35,36].

Woody plants growing in herbivore-dominated landscapes often have densely branched architectures[37,38], which decrease large mammal feeding rates[39] and increase the chances of survival under high herbivory pressure[40]. Dense branching can be attained by producing a

high number of shoot types or 'axis categories', e.g. trunk, branches, branchlets, brachyblasts (i.e. stems having very short internodes and lacking wood), with distinct morphologies and function[41,42] and comprise a trait that is stable at species level. The presence of spines (from various developmental origins, e.g., modified stem apices, modified petioles, Fig. 1) can further reduce large mammal feeding rates[43,44], and has cascading effects on plant biomass allocation, photosynthetic organisation and ontogeny[45,46,47]. Plants can achieve cage architectures through dense branching alone, and spiny species can have sparse, non-cage, architectures[48]. However, combining dense branching with spines likely produces the most effective herbivore defences, as dense branching translates directly into a higher spine densities.

Here we analyse how two traits, branching density and spine presence, evolved through deep time and in response to herbivory regimes, in a globally widespread plant family, Combretaceae. This family is well-suited to studying the evolution of plant-herbivore interactions as it is relatively modest in size (about 500 species) allowing substantial species coverage, displays a wide range of habits (lianas, shrubs, and trees[47]), and occupies numerous biomes[49,50] from closed canopy rainforests to savannas, where its species often dominate[;51,52,53].

We hypothesised that: (H1) Dense architectures and spines occur on species found in habitats that are subject to high herbivory pressure; (H2) Dense architectures and spines evolved independently; and, (H3) Clades possessing dense architectures and spines had significantly higher speciation rates under herbivory pressure than those without (Fig. 1). To test H1, we identified species developing in areas with high herbivory pressure based on the richness of large browsing herbivores and pre-Anthropocene herbivore biomass[24]. We then described branching density and spine presence in 132 Combretaceae species, and compared how the traits were expressed across herbivory regimes and biomes. To test H2, we estimated ancestral herbivory regimes and the timing of trait acquisitions through biogeographical inference, identifying potential congruent events. Lastly, H3 was examined using State-dependent Speciation and Extinction models (SSE framework[54,55]) to evaluate how dense architectures and spines influenced species evolution rates under different herbivory pressures.

RESULTS

Distribution of herbivory regimes

Global herbivory regimes were inferred by collating herbivore distribution ranges and calculating continent-level relative species richness (Supplementary Fig. S1 & S2). Cluster analyses (Fig. 2) then enabled us to identify which Combretaceae species occurred in: 1) herbivore-poor environments only (cluster A: low herbivory regime), 2) both high- and low-herbivory environments (cluster B: intermediate herbivory regime), or 3) herbivore-rich environments only (cluster C: high herbivory regime). Thresholds of mean values for low and high herbivore relative species richness were estimated at 29% and 58% respectively (Fig. 2).

Comparing species distribution in the different herbivory regimes with their occurrence in the biomes identified by Maurin et al.[49] shows a significant association of herbivore regimes with biomes, and with the canopy openness (Fig. 2E, Pearson's Chi-squared test, p-value = 0.046). High herbivory regimes mostly occur in dry savannas and eutrophic and mesotrophic woodlands and forests; intermediate herbivory regimes mostly occur in dry savannas, and mesotrophic woodlands and forests; and low herbivory regimes are more common in humid savannas and mesotrophic and dystrophic forest sand mangroves. These patterns are consistent with the fact that the abundance and richness of mammal herbivores is higher in open biomes[56]. The herbivore richness also increases in biomes with higher soil fertility where the nutritional content of leaves increases[57] and where Combretaceae fruits are more frequently vertebrate-dispersed[49].

Current structural diversity

When describing Combretaceae species architecture[42], we identified varied levels of architectural complexity. Four architectural strategies were found within our species pool (Fig. 1, Supplementary Fig. S4 & Table S1): 1) sparse non-spiny architectures, characterised by few axis categories, such as suffrutices; 2) sparse spiny architectures, characterised by few axes categories and possessing spines which are often petiolar, comprising mostly lianas[47], 3) dense architectures without spines, characterised by several axis categories and developing mainly as trees, and 4) dense and spiny architectures, characterised by several axis categories

and which are mostly shrubs. All possible combinations of the two traits were found in Combretaceae suggesting that both traits can be acquired independently.

Defensive traits associations with environment

The phylogenetic generalised linear regressions and phylogenetic ANOVAs performed on the density of architecture and spinescence, suggest that Combretaceae species with ranges restricted to high herbivory environments in Africa and globally are more densely branched and more frequently spiny (Fig. 3, Supplementary Table S4). Furthermore, the proportion of species with dense branching increases as herbivory pressure increases (Fig 3A). A similar pattern is observed for spiny species, significantly more abundant in environments with high herbivory pressure. Combretaceae species with dense or spiny architectures are thus more common where herbivory pressure is high, suggesting that resource allocation in environments exposed to high herbivory prioritises investment towards defence over resource acquisition[58,59].

Ancestral environment: biome and herbivory regime

Our ancestral herbivory regime estimation, for which the best fitting model is the trait-independent model, suggests that the ancestral habitat of Combretaceae had low to intermediate herbivory pressure. While several species display edible fruit for mammals[49], which are specifically distributed in eutrophic areas where mammal herbivores are more abundant[57], our results indicate that fleshy fruits did not affect dispersal rates between herbivory regimes. Only a few shifts in the herbivory pressure levels from low/intermediate to high are suggested, which occurred between 35 Mya (39-6 Mya, 95% confidence interval, "CI" hereafter) and 20 Mya (24-5.7 Mya, 95% CI; Supplementary Fig. S5-S9), consistent with inferred diversification events of large mammal herbivores during late Miocene[22,60]. Combretaceae's strong biome conservatism in open habitats[49] suggests that species may have experienced an increasing pressure of mammalian herbivory during the period in which they rapidly diversified. These changes in the intensity of environmental constraints possibly modified the selective pressure on plants, and could have influenced the direction of trait evolution, particularly those related to defence against herbivores.

Acquisition of structural defences

Ancestral states estimation under HiSSE model suggest that the structure of Combretaceae most recent common ancestor was likely neither densely branched nor spiny (Fig. 3, Supplementary Fig. S10 & S11). Dense architecture was acquired at least four times with the first acquisition at ca 60 Mya (62.7-20 Mya, 95% CI), and spines appeared at least seven times with the first acquisition dated to 20 Mya (323-6 Mya, 95% CI). Several reversions, from dense architecture to sparse architecture, are also suggested but increasing the sample size might lead to the estimation of fewer acquisitions. At least three of dense architecture acquisition events are linked in time with an environmental shift to herbivory regime (Fig. 3). The lineages which experienced both dense architecture acquisition and an environmental shift to high herbivory regimes seems to have produced more species than other lineages, suggesting the interaction of this trait with herbivory pressure might have been a driver of speciation, and prevented extinction of these lineages, conversely to species unable to defend against high herbivory pressure.

Herbivory regimes and speciation rates of dense species

Diversification analyses under SSE methods suggests that Combretaceae in Africa and globally had different speciation rates for different levels of caginess and herbivory pressure (Fig. 4, Supplementary Table S8 & S9). The best fitting model in both the African and global contexts included an interaction between branching density and herbivory regime. Under both the MuSSE and MuHiSSE frameworks, higher speciation rates (models best-fitting in 92.4% and 93.3% of 1000 phylogenetic trees sampled, Supplementary Table S10-15) were associated with dense architectures in environments with intermediate and high herbivory pressure (Fig. 4). Lineages with dense architectures occupying high herbivory environments experienced their highest speciation rates from 10 to 5 Mya (Supplementary Fig. S12). This coincides with the proliferation of browser species during the Late Miocene-Pliocene epoch (ca 12-5 Mya)[61], which might have provided an opportunity for speciation of densely branched plants that were better suited at resisting herbivory, especially because these species were less prone to disperse[49] in less pressured biomes. Possession of a highly branched architecture under high herbivory conditions, even without spines, might thus have been a

key confluence (sensu Donoghue and Sanderson[19]), suggesting a strong influence of large mammal herbivory as an evolutionary driver of vegetation.

Herbivory regimes and speciation rates of spiny species

Diversification models in spiny plants differed for Africa-only and global analyses. In Africa, the best fitting model included only an additive effect of spines (Fig. 5), and no effect of herbivory level (92.5% of the 1000 trees, Supplementary Table S16-18). By contrast, at global-scale, the best fitting model included the interaction between spines and herbivory regime (85.1% of the 1000 trees). The MuSSE and MuHiSSe frameworks both suggest that speciation rates were highest for spiny species occurring in environments with high herbivory pressure (4th highest speciation rates, Fig 5). These results suggest that a second key confluence might have arisen where spiny plants have encountered high herbivory conditions. The lack of support for this spines-herbivory regime interaction in African ecosystems may be the result of multiple origins of spines from different plant organs (e.g. leaf petioles, stem apices, lateral buds[62,63]), some of which appear to support climbing function. Coding each spine type origin separately might reveal key confluences in Africa. In addition, analysing a wider set of plant families would increase sample size and provide sufficient statistical power to test for key confluences in Africa, and may allow the identification of spiny architectures that evolved with and independently of dense architectures (e.g. trunk spines[47,64]).

Stepwise acquisition of cages

Our ancestral state estimations suggest that spine acquisitions mostly followed dense architecture acquisitions, with a single case of spine acquisition in a sparse structure (Fig. 3). This indicates that spines seem more likely to have emerged in an already complex architecture with many shoot types (i.e., axis categories), which suggests that the acquisition of defensive architectures likely occurred via a stepwise evolutionary process (Fig. 6): starting with an undefended ancestor, clades first acquired a densely branched architecture in evolutionary landscapes with intermediate herbivory (Fig. 3), which might have provided those species with an increased defence against vertebrate herbivores. The subsequent

acquisition of spines might have allowed clades to diversify (Fig. 7) and expand their ranges across landscapes with consistently high herbivory pressures.

Diversification analyses with both MuSSE and MuHiSSE indeed suggest that clades with both high branching density and spines have high speciation rates (best fitting model in 58.9% and 85.9% of phylogenetic trees sampled respectively, Supplementary Table S19-S21). In Africa, speciation rates for densely branched and spiny species were not significantly higher than for dense and non-spiny species, whereas at global scale, the interaction between dense architectures and spines produced the highest speciation rates (Fig. 7). Speciation rates show a peak at approximately 12 Mya, and a decrease from 5 Mya until present (Supplementary Fig. S12).

Thus, our results suggest that the acquisition of spines in an already densely branched architecture is likely a synnovation (sensu Donoghue and Sanderson[19]): while being independently acquired, the two traits might have had an interactive effect on speciation rates (Fig. 7). In Combretaceae, many of the spines originated via apex lignification of brachyblasts, which suggests that this functional specialisation might represent an exaptation of woody shoots from their initial role in assimilation to support the defensive function. The stepwise specialisation of architectures demonstrates that branching density should not be seen only as a mechanism for increasing the number of leaves or flowers that a canopy supports, but also as a component of large mammal herbivore defences. Additional architectural traits, e.g. branching mode, can also affect species selection under environmental constraints[12] and might have played a role in structural defences evolution.

DISCUSSION

Limitations and further steps

Using modern day relative herbivore species richness as a proxy for past herbivory regimes has limitations, as extinctions during the Pleistocene[65], most notably in South America and Australia, as well as anthropogenic modification of extant species distribution ranges (more specifically in Asia), will both have distorted this proxy measure. Nonetheless, relative herbivore species richness correlated closely with estimations of past herbivore biomass in sub-Saharan Africa (R^2=0.97, Supplementary Fig. S13)[29], which suggests that richness maps may well be useful for identifying large scale patterns of herbivory, and more precisely, habitat suitability for supporting large mammal herbivore communities. Similar validations

were not possible for South America and Australia, and results should be interpreted with caution. Plant functional traits associated with herbivory could further help deciphering the evolutionary drivers of biomes and help identify herbivore-derived ecosystems across the globe[23,24,25,43]. Future research would also benefit from exploring the global distribution of the structural traits examined in this study, dense architecture and spinescence, in relation to herbivory pressure in various plant clades. Human activities resulted in the extirpation or massive reduction in herbivore diversity in most parts of the World[66,67]. The recent removal of herbivore species from the habitat of structurally defended plants, might influence their ecological performance, potentially endangering them and making them prioritised targets for conservation.

Here we considered spines as a binary trait, which masks the varied investment in spines as ontogeny interacts with resource limitations[30,45,59,68] and in response to herbivory pressure. Induced defences, which are expressed only after injuries, were not considered in this study, and have been suggested to form an evolutionary step in the transition from plants being non-spiny to obligate spiny[59,69], while also optimising deployment costs under intermediate herbivory regimes[70,71]. Similarly, trunk thorns (Supplementary Fig. S14c) may have played a role in defence against climbing herbivores[47] which are more abundant in close canopy forests. In forests, petiolar spines (Fig. S14d-e) might also have supported the colonisation of new habitats, where plants experience a strong competition for light. These petiolar spines are often forming hooks, promoting anchorage in climbing plant species. Further research would be required to investigate whether these hooks should be considered an exaptation, with a shift of function from defence to climbing function, and potentially associated with an environmental shift from open to closed environments. Additionally, investigating how the structural defences we assess here trade-off with chemical defences[72,73,74] may help to explain further some of the patterns observed in this study[75,76,77]. For example, there are few, but still extant, species with sparse architectures in savannas with high herbivory pressure. These species-poor lineages might illustrate depauperons cases: while showing low speciation rates, a lineage still persisting without extinction over a long time. The acquisition of chemical compounds detering mammals to feed on leaves might have allowed this species to maintain extant in this habitat.

Lastly, we believe that investigating additional plant clades displaying a similar pantropical distribution will help to assess the generality of the patterns observed in this study. For

instance, Fabaceae, which have evolved the capacity to assimilate atmospheric nitrogen through root nodulations, are know to be highly nutritive for mammals[78] and would be an interesting candidate for studying how the parallel acquisition of nodulations, together with structural defences, played a role in species diversification and structural evolution in this clade.

Conclusion

Large mammal herbivory likely greatly influenced the evolution of plant architectures and lineage speciation rates in Combretaceae. Early clades diversified under low-intermediate herbivory regimes, with the acquisition of a densely branched architecture first promoting speciation under high herbivory pressure, and the subsequent acquisition of spines then further increasing speciation in high herbivory environments. Both traits are suggested to have been acquired as key confluences and interacted as a key synnovation, with the blocking defence of dense branching augmented by the sting of spines. While branching has often been assumed to be associated with light acquisition, here we show that large mammal herbivores are a potentially important factor to understand the step-by-step evolution of defensive cage architectures, and suggest a strong effect that large mammals have had on world flora.

Methods

Taxa sampling and description

We used the most recent, complete and accurate phylogenetic tree of Combretaceae[49] covering 30% of the species assigned to the family. This tree was based on a target capture approach using the Angiosperms353 probe kit[79,80]. We analysed species' architecture using photos linked to herbarium specimens or literature citations from the Global Biodiversity Information Facility (GBIF)[81], iNaturalist[82] and Plants of the World Online (POWO)[83]. A minimum of five different photographs were used per species. We were able to source photographs for several more species through contacts and our own field collections. Finally, the architecture of several species was described directly in the field from living specimens (14 species, which were described in Xishuangbanna Tropical Botanical Garden, China). We retained only photos taken in natural habitats displaying all selected architectural descriptors (see Halle et al.[84] and Barthelemy & Caraglio[42]) and having available geolocation data. We compiled data for a total of 132 out of the 161 species represented in the Combretaceae phylogenetic tree (26% of the Combretaceae species, Supplementary Table S1). We used the collated architectural descriptions to process the caginess index both numerically and categorically (dense: yes or no). The density index was measured according to Charles-Dominique et al.[37], based on the number of axis categories and the conicity of each axis (which indicates how rapidly wood develops). Spines (presence or absence of spines) were coded based on the presence/absence of sharp tips on one or more organ types (i.e. stem, leaf, stipule, epidermis).

Herbivory regimes assessments and relationships with traits

All analyses were conducted in R[85]. We first evaluated whether Combretaceae species co-occur today with large herbivores. We extracted the current distribution from the IUCN Red List database[86] for 5,843 terrestrial mammal species. We retained only animals most likely feeding on woody plants and affected by caginess and spines. We retained 265 species described in the literature as predominantly folivores (feeding on leaves, excluding frugivores and grazers), large sized (mass > 5kg) and terrestrial (excluding arboreal species). Non-ruminant megaherbivores (as categorised by Hempson[29]) were excluded as they were shown to not influence spines evolution[43]. The list of retained mammals, with references to their diet, is available in Supplementary Table S2. We extracted the worldwide occurrences of

each of these 265 herbivores in their natural distribution range as defined by IUCN[86] and rarefied them to a resolution of 0.05° (3 min square). We stacked presence records across species to obtain the herbivore richness per cell. The herbivory levels are very different across continents due to different land uses and pressure on wildlife. We divided herbivory richness by the continental maximal value to contrast areas with high or low levels of herbivory at the continental scale (maps of herbivory richness and herbivory relative richness are available in Supplementary Fig. S1 and S2; corresponding R script available in Supplementary Script S1).

We extracted occurrence data for all described Combretaceae species available in GBIF[61] using the package RGBIF[87], removed duplicates within species, and used the COORDINATECLEANER package[88] to remove problematic records and outliers (i.e. zero coordinates, country capitals, country centroids, biodiversity institutions), which resulted in the removal of 2% of the records. These data were also rarefied to 0.05° cells. We excluded species with four or fewer occurrence records from the analysis.

For each retained Combretaceae species occurrence, we extracted the herbivore specific richness, and used this information to estimate the minimum, mean, and maximum herbivore specific richness for each Combretaceae species.

We firstly only considered African clades (71 species) to describe the herbivory regimes. This was because Africa still retains a high mammalian herbivore richness and a wide distribution of those species, whereas mammalian diversity on other continents suffered high extinction during the Pleistocene. It also allows us to compare herbivore specific richness per grid to historical browser abundance, which aims to evaluate the strength of herbivore richness distribution; using Africa, we can make this comparison with a strong data set based on biomass[29].

We identified the main regimes influencing the Combretaceae distribution analysis on the [herbivore species richness (mean, minimum and maximum) × Combretaceae species geographic occurrences] matrix with Ward agglomerative clustering on Euclidean distance[89] (Supplementary Fig. S3).

Traits distribution across herbivory regimes

We tested whether dense architectures and spines differed across identified herbivory regimes identified, and also across the biomes identified by Maurin et al.[49] using the phylogenetic generalised linear regressions (function phyloglm, package "phylolm[90]") and phylogenetic ANOVAs (package "geiger"[91]). All the tests between traits (dense architecture and spines), and environment (herbivory regimes, biomes and canopy openness) has been performed on the 1000 trees, and indicated the mean p-value for the 1000 models. We additionally ran phylogenetic ANOVA on the 1000 trees, and similarly processed p-values. All results of the 2000 models (phyloglm and phylogenetic ANOVA) for dense architecture and spines are available in the Supplementary Material output folder (link). We additionnaly used Tukey's HSD test[92,93] for pairwisecomparisons of traits across the different herbivory regimes. The p-values from the tests were reported in Supplementary Table 4).

Ancestral state and range estimation

We analysed whether the evolutionary rates associated with caginess and spines differed among the different herbivory regimes identified.

We mapped the ancestral states for caginess and spines, using the "fitHRM" function in the PHYTOOLS package[94], performing stochastic mapping and summarising character state probabilities at each node. fitHRM implements the hidden-rates trait evolution model of Beaulieu et al.[55].

We estimated ancestral herbivory regimes using the BioGeoBEARS package[95], according to the method of Klaus & Matzke[96]. We tested whether better fitting values were obtained by adding all relevant parameters to the model (founder events parameter, j, transition rates of the dispersal mode, t12 + t21, and the modifier on dispersal rates, m2), and retained the model with the best-fitting AIC (Supplementary Table S4 to S6). We followed the method for model selection provided by Klaus & Matzke[96] (but see more details in Supplementary Notes S1). We used the initial transition rates (t12 and t21) for animal-dispersed seeds as estimated by Maurin et al.[49]. We performed stepwise selection for DEC, DIVALIKE and BAYARELIKE models. We fixed the modifier on dispersal rate under state 1 (m1) to the value of 1 (state 1 is state "yes", and state 2 is state "no"), and set the modifier on dispersal rate under state 2 (m2) as free, following Klaus & Matzke[96] on the retained tree topology by Maurin et al.[49].

Evolution rate estimates with MuSSE

We performed multiple state speciation and extinction (MuSSE) analyses using the package DIVERSITREE[97] to evaluate evolution rates of caginess (categorical), spines (categorical), and herbivory regimes (categorical). The branch density index was processed as categorical (yes/no) using a threshold based on the mean and the median of the index (which both gave similar results), as MuSSE analyses allow the modelling of evolution rate for multiple binary traits, and quantify the effect (additive or interactive) of two binary traits together. This allowed us to test the hypotheses that 1) caginess and 2) spines impacted evolution rates if species were growing under high herbivory pressure (interaction of dense architecture and herbivory, spines and herbivory), and 3) that spines improve cage efficiency through evolutionary rates (interaction of dense architecture and spines). For each association of binary traits, we analysed the data for two datasets, Africa only and worldwide. We tested 17 models (Supplementary Table S7) for which we estimated the AICc and ΔAICc to evaluate which was the best fitting. Each model was tested over 1000 phylogenetic bootstrap trees (used for stochastic mapping) obtained from the concatenated analysis of the Angiosperms353 loci produced by Maurin et al.[49] and dated using treePL[98]. We summed the instances in which each model was best fitting and selected the one with the highest number (Supplementary Table S8 to S13). When a trait-dependent model had a better fit than the null model (trait independent evolutionary rates), we ran 10,000 MCMC steps (keeping the 95% credible interval) using "an exponential prior with a mean set to twice the state-independent diversification rate" (2r) (FitzJohn 2012), and a sampling proportion of 0.26 (global) and 0.14 (Africa only). MCMC outputs are available in Supplementary Table S14 to S19. The 17 models tested do not include free extinction rates parameter (mu) as letting this parameter varying can drastically increase Type I errors (false positives)[99]. The models tested include: 1) the null model, where traits does not affect speciation rates and transition rates, 2) models allowing rates to vary with an additive effect of traits on the speciation rates only, on the transition rates only and on both speciation rates and transition rates, and 3) models allowing rates to vary with an interaction effect of traits on the speciation rates only, on the transition rates only and on both speciation rates and transition rates. A script allowing to perform the whole analysis with parallel computing is available (Supplementary Script S2).

Evolution rates estimates with MuHiSSE

Where evidence of state-dependent effect on speciation rates was detected in the MuSSE framework, we further investigated the possibility that the effect observed on rates was also dependent on one or several hidden states, which are states undescribed. In this order, we used the Multistates Hidden State Speciation and Extinction framework (MuHiSSE)[100] to address concerns about the Type I errors often occurring with BiSSE and MuSSE[97]. We fitted seven additional models using the MuHiSSE framework, adding from two to eight hidden states, which is the maximum allowed by the framework. The models tested include: the null model, where traits do not affect the net diversification rates, models allowing diversification rates (but no extinction rates) to vary with an interaction effect of traits (MuSSE model), and seven models adding from two to eight hidden states on the MuSSE model (MuHiSSE). All models were tested over 1000 phylogenetic trees sampled from a set of bootstrap trees. This allowed us to test whether the effect of a trait on evolution rates (speciation and transition rates) potentially detected in MuSSE framework was mostly explained by the traits themselve, or by a random effect. A script allowing to perform the whole analysis with parallel computing is available (Supplementary Script S3). We further extracted the turnover rates (speciation and transition rates, without including extinction rates) inferred at each node and plot these rates over time using the MarginReconMuHiSSE function of the hisse package[101].

Reporting summary

The authors declare that availability of data, materials, code and protocols comply with Nature Springer Journals & Nature Portfolio standards.

Data Availability statement

The descriptions data (cagey index and spines), the herbivory richness data and outputs for all analyses that support the findings of this study have been deposited in Figshare with the doi: https://doi.org/10.6084/m9.figshare.24454609

The additional datasets used in this study are GBIF datasets (GBIF.org, 27 October 2022, GBIF Occurrence Download: https://doi.org/10.15468/dl.7p2kpa) and the IUCN RedList Database (IUCN. 2022. The IUCN Red List of Threatened Species. Version 2022-2. https://www.iucnredlist.org.)

Code Availability statement

The R code used for all analyses of this study have been deposited in Figshare with the doi: https://doi.org/10.6084/m9.figshare.24454609

Author contributions statement

AA, TCD and KWT designed the project; AA conducted data collection and processing; AA and YBK performed the analyses; AA, YBK, TCD, YC, FF, GPH, OM and KWT wrote the manuscript.

Competing interests statement

The authors declare no competing financial interests.

Figure Legends

Fig. 1 : Traits studied and hypotheses tested. (1) Number of axis categories has been processed according to Barthelemy & Caraglio[50], allowing to characterise the density of architecture based on the cage index (removing spines) developed by Charles-Dominique et

al.[36] (but see more details in Method). (2) Spinescence has been scored as yes/no independently of the spine ontogenetic origin. Under hypothesis H1, dense architectures and spines should have an environmental-dependent distribution, specifically varying under different levels of herbivory pressure. Under hypothesis H2, the two trait acquisition(s) are independent from each other; and under hypothesis H3, herbivory pressure have played a role on trait selection and reflect on speciation rates of species.

Fig. 2: Global herbivore richness, herbivory regimes and their relationship with plant defensive architectural traits, and ancestral state and herbivory regime inferences. A: Relative herbivore richness per grid square (0.05 x 0.05 degree grid square); the richness of every grid square has been divided by the maximum richness of the continent on which it occurs, to obtain the relative richness per grid square per continent. B: Clustering of herbivory regimes: the three clusters separates areas with low herbivory regime (A) intermediate herbivory regime (B) and high herbivory regime (C); every point represents a Combretaceae species and its attributed herbivory regime. C: Herbivore relative richness according to Combretaceae occurrences in each cluster (n = 132 different Combretaceae species) box and whisker plots show the median, 1st and 3rd quartiles, and bounds of the herbivore relative richness per regime. C: Herbivore proportions according to Combretaceae occurrences in each cluster. D: maps of the herbivory regimes assigned to Combretaceae species; E: Biomes and canopy openness (from Maurin et al.[48]) associated to each herbivory regime, and their associated Two-tailed Pearson's chi-squared test (n = 132 different Combretaceae species); bar plots show the cumulative percentages of the biomes and canopy openness in the different herbivory regimes.

Fig. 3: Plant defensive architectural traits in association to environmental factors, ancestral state and herbivory regime inferences. A: Current plant trait distribution in the different herbivory regimes and level of canopy openness; stars indicate the significant positive association (respectively p-value = 0.001, 0.001, 0.020, 0.021) according to phylogenetic generalised linear regression and two-sided phylogenetic ANOVA (Supplementary Table 4) and grouping letters show the result of pairwise comparisons (Tukey's HSD tests with n = 132 different Combretaceae species; box and whisker plots show the median, 1st and 3rd quartiles, and bounds of the trait distribution in herbivory regimes

and according to canopy openness levels). B: Ancestral state, herbivory regime and biome inferences; the colours on the branches represented the attributed herbivory regime, with intermediate states (see Supplementary Fig. S8 to S9); the pies on the nodes show major changes in acquisition of dense architecture (yellow) and of spines (red); only transitions with a probability > 0.75 are shown; close canopy (blue pentagons) and open canopy (yellow triangles) summarise information from Maurin et al.[48].

Fig. 4: Speciation rates of species with dense architectures under herbivory regimes according to the best fitting models in the MuSSE framework. Rates were processed using 10,000 MCMC generations on 1000 trees. A- Rates for the models considering Africa only (box and whisker plots show the median, 1st and 3rd quartiles, and bounds of the 95% Bayesian credibility intervals of the speciation rates in herbivory regimes) B- Rates for the models at the global scale (box and whisker plots show the median, 1st and 3rd quartiles, and bounds of the 95% Bayesian credibility intervals of the speciation rates in herbivory regimes). The best fitting models for dense architecture are the models including an interaction effect between dense architecture high herbivory regime (coloured); Source data corresponding to MCMC outputs are available in Supplementary Material (output folder).

Fig. 5: Speciation rates of spiny species under herbivory regimes according to the best fitting models in the MuSSE framework. Rates were processed using 10,000 MCMC generations on 1000 trees, at the global scale. The best fitting models for spiny species are the models including an interaction effect between spines and high herbivory regime (box and whisker plots show the median, 1st and 3rd quartiles, and bounds of the 95% Bayesian credibility intervals of the speciation rates in herbivory regimes). Source data corresponding to MCMC outputs are available in Supplementary Material (output folder).

Fig. 6 Combretaceae architecture evolution under different herbivory regimes. This schematic representation illustrates a single transition from sparse to dense architecture, followed by two transitions from non-spiny to spiny. At least three of these stepwise acquisitions of traits have been observed in Combretaceae. Grey framework represents a simplified phylogeny, the thickness of the grey area represents the speciation rates. Coloured

circles on the phylogeny indicate trait acquisition; coloured squares on the phylogeny show shifts of herbivory regime (from low-moderate to high herbivory regime); dotted lines indicate the time of appearance (on the left) of trait and shift of herbivory regime. Light colour area on the background shows the confluence between dense architecture and high herbivory regime, the darker background shows the synnovation between dense architecture and spines.

Fig. 7: Speciation rates of spiny species with dense architectures according to the best fitting models in the MuSSE framework. Rates were processed using 10,000 MCMC generations on 1000 trees, at the global scale. The best fitting models for spiny are the models including an interaction effect between spines and dense architectures (box and whisker plots show the median, 1st and 3rd quartiles, and bounds of the 95% Bayesian credibility intervals of the speciation rates in herbivory regimes). Source Source data corresponding to MCMC outputs are available in Supplementary Material (output folder).

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