Spiny plants, mammal browsers, and the origin of African savannas

Tristan Charles-Dominique[a,b,1], T. Jonathan Davies[c,d], Gareth P. Hempson[e], Bezeng S. Bezeng[c], Barnabas H. Daru[f,g], Ronny M. Kabongo[c], Olivier Maurin[c], A. Muthama Muasya[a], Michelle van der Bank[c], and William J. Bond[a,h,1]

[a]Department of Biological Sciences, University of Cape Town, Rondebosch 7701, South Africa; [b]Department of Botany and Zoology, Stellenbosch University, Matieland 7602, South Africa; [c]African Centre for DNA Barcoding, Department of Botany and Plant Biotechnology, University of Johannesburg, Johannesburg 2006, South Africa; [d]Department of Biology, McGill University, Montreal, QC, H3A 0G4 Canada; [e]School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Wits 2050, South Africa; [f]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138; [g]Department of Plant Sciences, University of Pretoria, Pretoria 0028, South Africa; and [h]South African Environmental Observation Network, National Research Foundation, Claremont 7735, South Africa

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Savannas first began to spread across Africa during the Miocene. A major hypothesis for explaining this vegetation change is the increase in C_4 grasses, promoting fire. We investigated whether mammals could also have contributed to savanna expansion by using spinescence as a marker of mammal herbivory. Looking at the present distribution of 1,852 tree species, we established that spinescence is mainly associated with two functional types of mammals: large browsers and medium-sized mixed feeders. Using a dated phylogeny for the same tree species, we found that spinescence evolved at least 55 times. The diversification of spiny plants occurred long after the evolution of Afrotherian proboscideans and hyracoids. However, it is remarkably congruent with diversification of bovids, the lineage including the antelope that predominantly browse these plants today. Our findings suggest that herbivore-adapted savannas evolved several million years before fire-maintained savannas and probably, in different environmental conditions. Spiny savannas with abundant mammal herbivores occur in drier climates and on nutrient-rich soils, whereas fire-maintained savannas occur in wetter climates on nutrient-poor soils.

Africa | Bovidae | coevolution | mammalian herbivory | savanna

The origin and spread of savannas have been topics of intensive research, but many questions remain. The C_4 grasses that dominate savannas emerged in the late Oligocene (~30 Ma), but savannas only began to emerge as one of the world's major biomes in the late Miocene more than 20 My later (1). What changed to roll back the forests, allowing the rapid spread of grasslands? Ehleringer et al. (2) first linked the rise of savannas to a drop in atmospheric CO_2, which would favor C_4 grasses over their C_3 grass predecessors. Low CO_2 can also reduce woody cover by increasing the risk of recruitment failure in woody plants whether from drought, fire, or browsing (3). However, the timing of the onset of low CO_2 is much earlier than the spread of savannas; therefore, although low CO_2 may have contributed to savanna expansion, it cannot explain the long time lag between C_4 origins and savanna spread. Climate change is the usual explanation for changing vegetation over time. Increased aridity in the late Miocene has been shown to cause the retreat of forests in North America and Eurasia, allowing grasslands to spread in their place (4, 5). However, large areas of extant savannas occur in climates that are wet enough to support forests and other closed woody types (6–8). Fires are frequent in high-rainfall savannas and have been considered the major agents accounting for open ecosystems in climates that can support forests. Fossil charcoal, mostly from marine cores, shows a surge in fire activity from the late Miocene correlated with the spread of savannas (9, 10). Phylogenetic studies have shown the emergence of fire-adapted woody plants from the late Miocene through to the Pleistocene in both Brazil and Africa, consistent with fossil evidence for increasing fire activity from this time (11, 12).

An alternative hypothesis, that mammal herbivory creates open ecosystems, was first proposed by Owen-Smith (13). He argued that increased forest cover from the last glacial to interglacial conditions was partly the result of extinction of the Pleistocene fauna (13). Many experimental and observational studies have shown that mammals have the capacity to create open ecosystems by reducing tree biomass whether in the tropics or temperate and boreal regions (14, 15). Although Owen-Smith (13) emphasized megaherbivores (animals >1,000 kg) as primarily responsible for open habitats, mesobrowsers (4–450 kg), such as deer, antelope, and caprids, are very effective at preventing woody plants from escaping the "browse trap" and growing into larger size classes. They have also been implicated as agents maintaining open ecosystems and preventing forest development. Here, we explore the potential role of mesobrowsers in opening up ancient ecosystems and promoting the spread of African savannas. Africa is particularly suitable for such a study, because it retains a largely intact megafauna, although now greatly reduced in abundance and area.

Fossil tests of the importance of herbivory in opening up ancient forests are difficult because of the lack of sites with suitable data on both plant and animal fossils. This lacuna in the fossil record is explained partly because contrasting conditions favor fossil development in plants vs. mammals (16). For example, where plant fossils

Significance

Africa hosts contrasting communities of mammal browsers and is, thus, the ideal background for testing their effect on plant communities and evolution. In this study at the continental scale, we reveal which mammal browsers are most closely associated with spiny communities of trees. We then show a remarkable convergence between the evolutionary histories of these browsers (the bovids) and spiny plants. Over the last 16 My, plants from unrelated lineages developed spines 55 times. These convergent patterns of evolution suggest that the arrival and diversification of bovids in Africa changed the rules for persisting in woody communities. Contrary to our current understanding, our data suggest that browsers predate fire by millions of years as agents driving the origin of savannas.

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Data deposition: The data reported in this paper are available in Dataset S1 and at www.boldsystems.org/, www.ncbi.nlm.nih.gov/genbank/, www.ville-ge.ch/cjb/, www.naturalis.nl/nl/, www.gbif.org/, worldclim.org/, climate.geog.udel.edu/~climate/, www.fao.org/home/en/, www.globalfiredata.org/, and modis.gsfc.nasa.gov/.

[1]To whom correspondence may be addressed. Email: tristancd1@gmail.com or william.bond@uct.ac.za.

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are well-preserved in acidic deposits, animal bones are not. Recently, paleoecologists have begun using the spores of a coprophilous fungus, *Sporormiella*, as a proxy for high herbivore activity. Fungal spores can be counted along with pollen and charcoal to determine changes in herbivory, fire, and vegetation (17). Such studies have suggested changes from open ecosystems with high *Sporormiella* counts to closed woody vegetation or in some cases, a switch to flammable vegetation when the dung spores decline (18, 19). Thus, there is growing paleoecological evidence that mammal herbivory helped maintain open ecosystems in the past and that large mammal extinction triggered major vegetation change. However, these studies have focused on the Late Pleistocene and Holocene, and the earlier origins of open grassy formations have been less explored (20).

Here, we report on a phylogenetic approach for exploring the importance of mammal browsers in opening up African vegetation and promoting the spread of savannas from the late Miocene. Although several phylogenetic analyses have explored plant defenses against insect herbivory, we report here an analysis of the evolution of tree defenses against vertebrate herbivores. We used stem (not leaf) spines on woody plants as markers of high mammal herbivory (Fig. S1). Spines are considered a defense specific to mammal herbivores (21). We did not include leaf spines or species with soft organs, stinging hairs, or spines shorter than 5-mm long, because their defensive function against vertebrate herbivory remains unclear. Spines are a peculiar defense in that the foliage of spiny trees is often highly palatable and favored by browsers. Spines function by reducing bite size of the browsing animal, thereby reducing food intake and driving the animal to move away to seek more rewarding targets (22). In this paper, we first establish how the present day distribution of spiny species relates to the abundance of different herbivore functional types, abiotic environmental factors (precipitation, soil fertility, fire, and temperature), and major vegetation types. We used a recent classification of African herbivores that groups together animals according to their functional traits (body mass, diet, gut type, social behavior, and water dependence) (23). We then explore coevolutionary dynamics by comparing the accumulation of spiny plant lineages in African savanna with the diversification of bovids (antelope and their relatives). Although bovids and mesobrowsers are not strictly equivalent, most mesobrowsers in Sub-Saharan Africa are bovids. The bovid lineage includes the browsers most related to spiny plant distribution today. A close match would imply a causal link between the diversification of bovids and spiny plants. If spines emerged much earlier than bovid radiations, then it is possible that these plants were preadapted to bovid herbivory, and it is unlikely that mammal herbivory was a major factor in their spread. Finally, we compared the timing of the increase in spiny plant lineages and the diversification of their mammal browsers (bovids) with phylogenetic and fossil evidence for the emergence of savannas.

Results

Contemporary Environmental Correlates. Our spatial model explained over 60% of the distribution of spiny communities in Africa (Fig. 1*A* and Table S1). Spinescence of vegetation was significantly related to the suite of herbivores present (adjusted $R^2 = 0.38$), abiotic factors (0.35), and the distribution of biomes (0.27). Spiny communities are favored in open environments, such as mixed savannas (all scores are reported in Table S2), shrubland, savanna–shrubland, and grassland–shrubland, and poorly represented in evergreen forest and forest/grassland mosaics (Figs. 1*B* and 2). The abiotic environments related to spinescence include low mean annual precipitation (Fig. 1*B*), high-nutrient soils, colder temperature, and low fire frequencies. Importantly, the unique contribution of herbivores (0.081) is significant even after removing the variation coexplained with abiotic factors or biome distribution. The relationship between spiny plant and herbivore communities is strong and positive for medium-sized mixed feeders (consuming grass and trees) and large browsers (Fig. 1*B*).

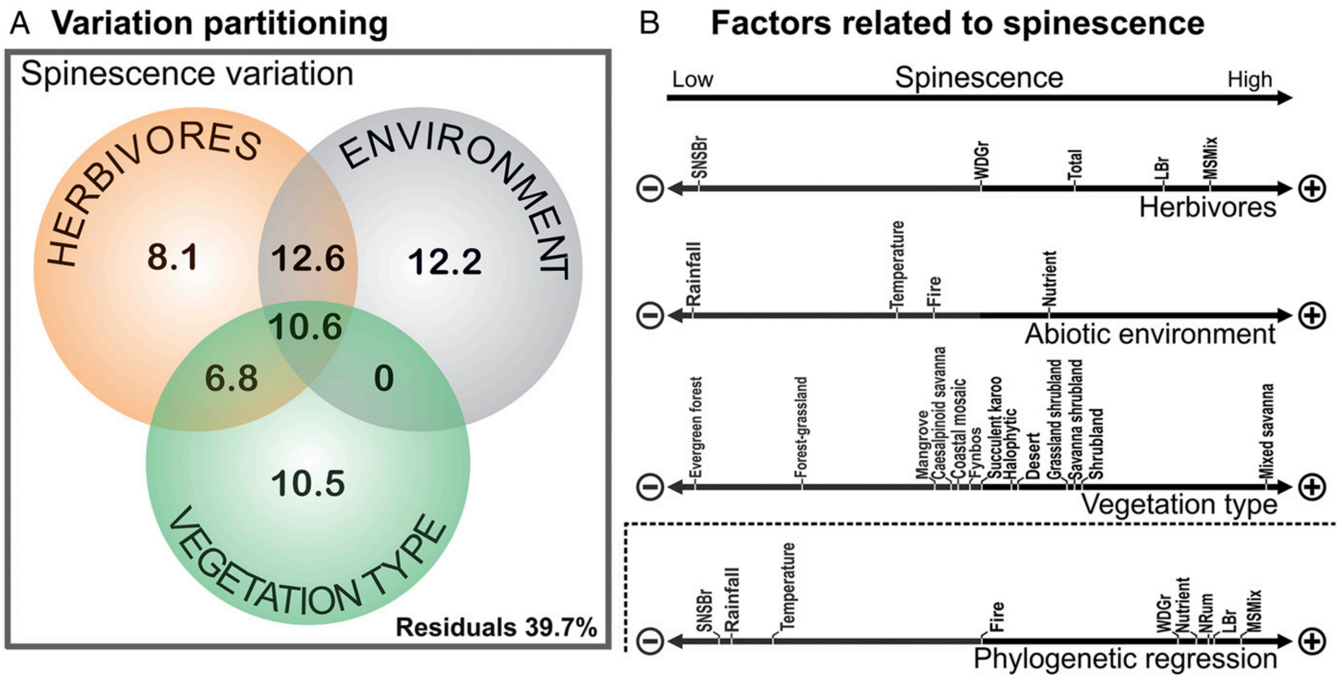

Fig. 1. Environmental factors related to spinescence of African tree species. (*A*) Variation partitioning of the proportion of spiny species explained by the biomass of different herbivore functional types, environmental variables, and vegetation type. (*B*) Relationships between environmental factors and proportion of spiny species. Black arrows indicate a positive relationship; gray arrows indicate a negative relationship. The position on the arrow indicates the strength of the relationship. Names of functional types of mammals follow the work in ref. 23. LBr, large browser; MSMix, medium-sized social mixed feeders; NRum, nonruminant; SNSBr, small nonsocial browser; Total, total biomass of mammalian herbivores; WDBr, water-dependent grazer.

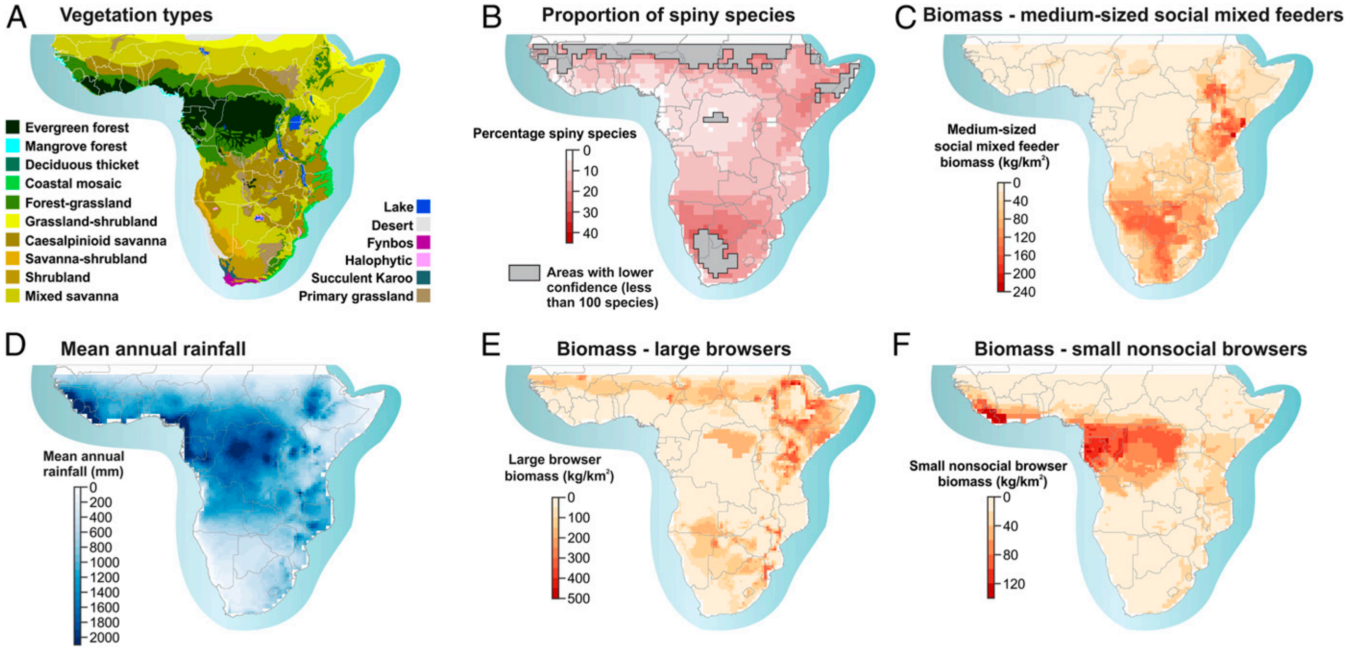

Fig. 2. Maps of spiny species distribution and environmental correlates. (*A*) Vegetation types. (*B*) Proportion of spiny species: values for areas in gray are not reported because fewer than 100 tree species have been reconstructed in each degree pixel; interpretation of proportions should then be subject to caution. (*C*) Total biomass of medium-sized social mixed feeders. (*D*) Mean annual rainfall. (*E*) Total biomass of large browsers. (*F*) Total biomass of small nonsocial browsers.

In contrast, spinescence is strongly negatively associated with small nonsocial browsers, which are largely restricted to rainforest (Fig. 2). The weaker relationship with the total biomass of herbivores emphasizes the usefulness of the functional grouping of herbivores to identify groups best associated with spinescence.

Phylogenetic regression on the principal components of the combined explanatory dataset identified similar important variables (Fig. 1*B*). The best model included only the first principal component (Table S3), which was positively related to spinescence ($P = 0.022$) (Table S4). Variable correlations with the first principal component (Table S2) suggest that spinescence is associated with dry, somewhat cooler environments with high soil nutrient status (all scores are reported in Table S4). Medium-sized mixed feeders, large browsers, nonruminants, and to some extent, water-dependent grazers are positively related to spinescence, whereas small nonsocial browsers have a strong negative correlation with spinescence (Fig. 1*B* and Table S4). Fire prevalence seems to have virtually no association with spinescence.

Phylogenetic Analyses. The distribution of spinescence in African trees is phylogenetically dispersed (Fig. 3). We recorded 213 spiny species (from a sample of 1,852 tree species) distributed in 29 families, indicating at least 55 independent evolutionary origins across the angiosperm tree of life (Dataset S1). Spiny clades have relatively recent origins, with the great majority arising within the last 17 Ma (Fig. 3) but with some older origins, frequently associated with clades that likely diversified elsewhere (Table S5). The mid-Miocene radiation of spiny plants indicates that early Cenozoic African herbivores, notably proboscideans (including elephants) and hyracoids, did not select for this structural defense.

Accumulation of lineages of spiny trees and bovids (data from ref. 24) (both bovid and tree phylogenies have multiple calibrations by fossils) is remarkably congruent in time (Fig. 4). The origin and timing in acceleration of bovid lineage diversification match closely to the increase in spiny plant lineages with present day descendants in African savanna. The suggestion of some apparently older dates for the origin of spines, perhaps as far back as 40 Ma, reflects uncertainty in character states deeper in time and the fact that some spiny taxa are nested within non-African clades (i.e., we are missing their close relatives) (Fig. S2 and Table S5). Our sampling of plant lineages only encompasses woody tree species with southern African distributions but is relatively complete for spiny taxa, and we have reasonable confidence that we are not missing species-rich spiny lineages with earlier origins.

Discussion

Are Spines a Good Proxy for Mammal Presence in Contemporary Savannas? We analyzed three linked explanations for the present day distribution of spinescent species in Africa: (*i*) growing conditions in which spines are more effective, (*ii*) carbon costs, and (*iii*) mammal herbivore pressure. In our analyses, spiny communities are associated with more arid and nutrient-rich savannas. These communities are the "eutrophic" savannas of Africa (25). These environments support high mammal biomass (23, 25) and high browser diversity (26). Spinescent species are rare in forest and negatively associated with its mammalian fauna. In contrast, open savannas have a high proportion of spiny species, suggesting that the costs of structural defenses (27) may be incompatible with low light levels found in forests. Our results constitute evidence at large spatial scales that the abundance of mammals is the factor best related to the distribution of spiny communities. Our results are not explained by covarying abiotic environmental factors or vegetation types and remain significant, even after controlling for these factors. Additionally, phylogenetic analyses show that herbivory has selected for the evolution of structural defenses in multiple woody plant lineages.

Using the functional grouping of mammal herbivores (23), we found that "medium-sized social mixed feeders" are the functional type most closely associated with spinescence. This group of species generally grazes more during the wet season and browses more in the dry season. Mixed feeding has several consequences. First, high-quality new grass growth can support

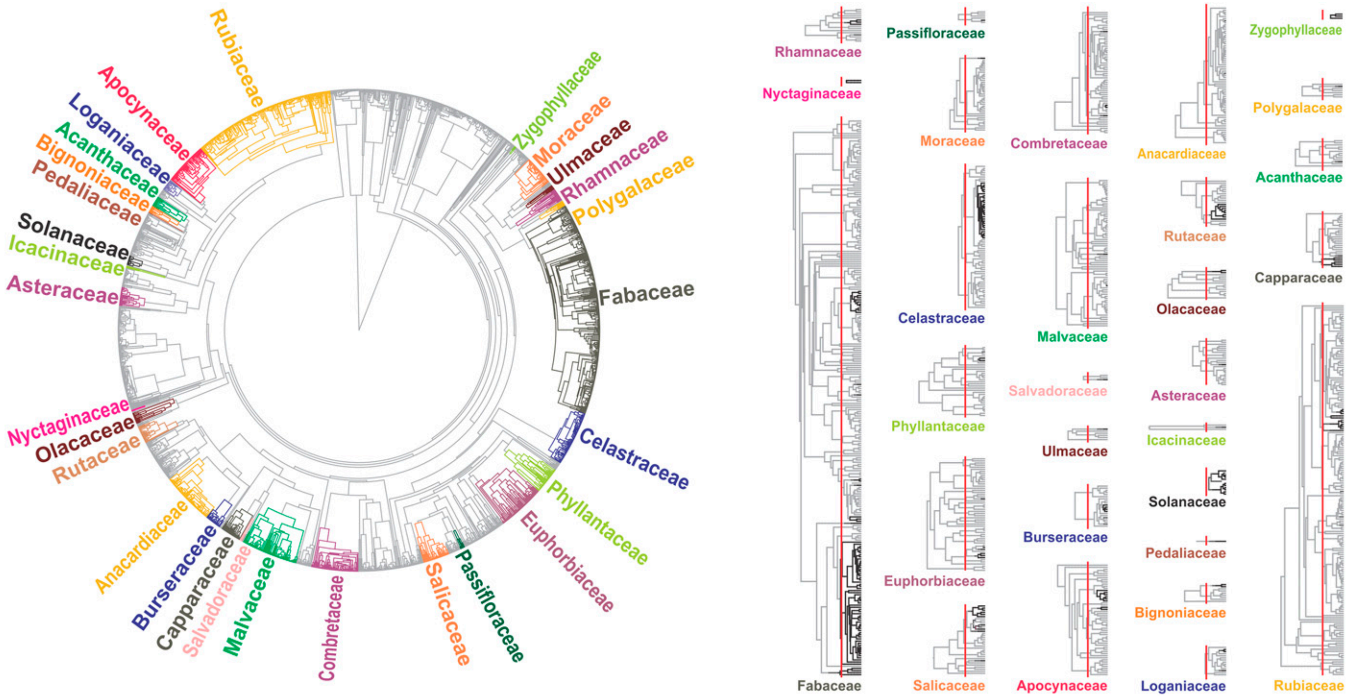

Fig. 3. (*Left*) Phylogeny of southern African woody flora and position of spiny species. Flora were reconstructed based on DNA barcodes using a maximum likelihood approach after transforming branch lengths to millions of years ago by enforcing a relaxed molecular clock and multiple calibrations. Colored branches highlight families containing at least one spiny species. The subtrees for these families are presented (*Right*), with the position of spiny species indicated in black (shades of gray indicate the probability of the ancestral lineages being spiny). The red bar indicates 17 Ma on all subtrees.

large herds of mixed feeders during the wet season, which then amplifies their selective effect on trees when these herds switch to browsing during the dry season; second, mixed feeder impacts on plant populations should be stronger where dry seasons are longer, as suggested by our results that show spinescence to be associated with more arid savannas.

Bovids and Spiny Trees: A Coevolutionary Relationship? Patterns of convergent evolution provide a test of adaptation between herbivores and plant defenses (28). We show that spinescence evolved independently multiple times in the phylogeny of African trees and many different families and was achieved by three different developmental pathways: stipular spines, epidermic prickles, and thorns. Character optimization on the dated phylogeny indicates that nearly all of the modern spiny species evolved since the early Miocene.

Before the Miocene, spinescence evolution could have been limited by unfavorable physical environmental conditions or their ineffectiveness against modes of feeding of early Cenozoic African mammals. The rarity of spines in modern forests could imply that a more forested environment in the Paleogene may have inhibited spine development. However, this argument is contradicted by the presence in Africa of thicket from the early Eocene (29). Thicket is a low, dense woody vegetation with intermediate light level between forests and savannas that supports a high diversity of spiny trees (29). The early appearance of thicket and the much later appearance of spiny plants in our analysis, thus, suggest that the evolution of spinescence is linked to the arrival of bovids in Africa during the Neogene.

Structural defenses vary with mode of feeding: for example, defenses against large bird browsers are quite different from those of "mammal" browsers (30). However, there are few studies of variation in defense structures in response to different modes of mammal feeding. Africa was an island continent from the beginning of the Cenozoic with an endemic African fauna, the Afrotheria, including the extinct rhinoceros-like embrithopods, Hyracoidea, and Proboscidea (of which hyraxes and elephants, respectively, are the sole extant examples) (16). Hyracoids were diverse, ranging in size from small rabbits to rhinoceros, and the ecological analogs

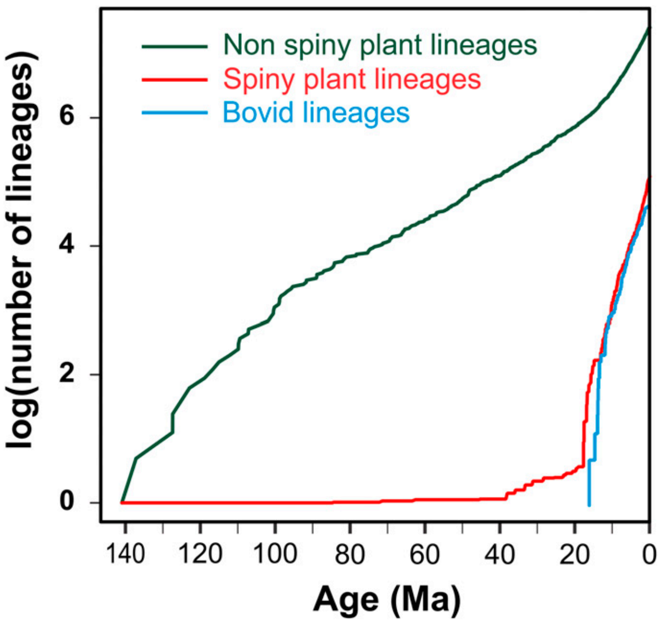

Fig. 4. Lineage accumulation through time of bovids and spiny and nonspiny southern African woody species. Information for the bovid lineages is sourced from ref. 24. Plant lineage richness for spiny and nonspiny taxa was estimated as the sum of the number of lineages multiplied by their probability for the respective character state.

of perissodactyls and artiodactyls on other continents (16). Apparently, none of these lineages selected for spines as a plant defense. Modern elephants, the last of the proboscids, have been documented as destroying stands of spiny tree species (31). A land bridge developed between Africa and Eurasia in the late Oligocene/Miocene, and Eurasian mammals (rhinoceros and suids) entered Africa for the first time. By mid-Miocene (16 Ma), there was a second invasion of Eurasian elements, including horned bovids and antlered giraffoids, with another wave of Eurasian immigrants in the late Miocene (16).

The diversification of bovids (24) from mid-Miocene closely matches the rapid accumulation of spiny plant lineages in African savanna (Fig. 4), suggesting that bovids presented a novel mode of feeding that spread in Africa after the Miocene and selected for the evolution of spinescence. Interactions between trees and mammals might have resulted in niche diversification for both groups. This suggestion is consistent with contemporary patterns of diversity identified for *Acacia* species (sensu lato; i.e., *Vachellia* and *Senegalia*) and mammalian herbivores (26). Several processes could be involved in niche diversification: (*i*) segregation of niches in height: herbivores have distinct feeding strategies (bite size and tolerated fiber content) depending on their body size (32), and the vertical deployment of spines on plant species has been shown to match the body size of herbivores present (33); (*ii*) niche specialization: mammals that browse more have narrower muzzles, longer tongues, and prehensile lips, allowing them to handle thorny plants better than grazers (22); and (*iii*) segregation of niches in time for trees: browser impacts vary with season, differentially affecting evergreen and deciduous tree species (34, 35).

The second functional type of herbivores related to spinescence ("large browsers") in modern Africa includes giraffes and okapi, two members of the Giraffidae family. Fossil giraffids were formerly more diverse and are known since the middle Miocene in Africa (16). Although not as abundant or diverse as bovids, giraffids might also have played a role in selecting for spinescence.

Bovids and the Spread of Savannas. The timing of increase in spinescence, which we have shown to associate with savannas, provides a proxy for dating the spread of mammal-dominated savannas independent of isotopic evidence (36, 37). The rise of savanna has been attributed to increased aridity promoting grasses over trees (4) or a drop in CO_2 concentrations promoting the spread of C_4 grasses (Fig. 5). Although these two factors define the climatic envelope suitable for grasses, they do not account for the exclusion of forests that could be supported along the precipitation gradient in Africa (38, 39). Simulations illustrate well that aridity and low CO_2 are not enough to explain the spread of savannas; an additional force opening up forests is needed (40). Fire is one likely causative agent (9, 41). Here, we have shown that herbivory pressure might provide another pathway. Our results indicate that the influx of bovids triggered savanna formation from the mid-Miocene, long before its explosive spread because of fire in the late Miocene. The precise dating of this ecological transition is difficult: molecular dating could overestimate divergence ages, and the use of fossils most often underestimates them (42). This problem is especially true in the African context, where the fossil record for trees and mammals is patchy and restricted to few localities that poorly describe the full ecological spectrum of the continent (4, 16, 43). However, we can compare relative dating using phylogenetic analysis of spines as a marker of mammal-dominated savannas and geoxylic suffrutices as markers of fire-dominated savannas (12). The same woody plant phylogeny shows an accumulation of spiny plant lineages in Africa several million years earlier than the appearance of geoxyles, suggesting that mammal-dominated savannas predate fire-dominated savannas by millions of years (Fig. 5). Moreover, savannas maintained by fire and herbivory seem to be favored in contrasting environmental settings: fire-dominated (dystrophic) savannas on seasonally humid and nutrient-poor environments vs. herbivore-dominated (eutrophic) savannas on arid and nutrient-rich environments (7, 23, 25). If these two types of savannas occurred elsewhere in the world, then Pleistocene mammal extinctions may only have had limited effects on releasing fire as an agent and then, only in eutrophic, semiarid savannas.

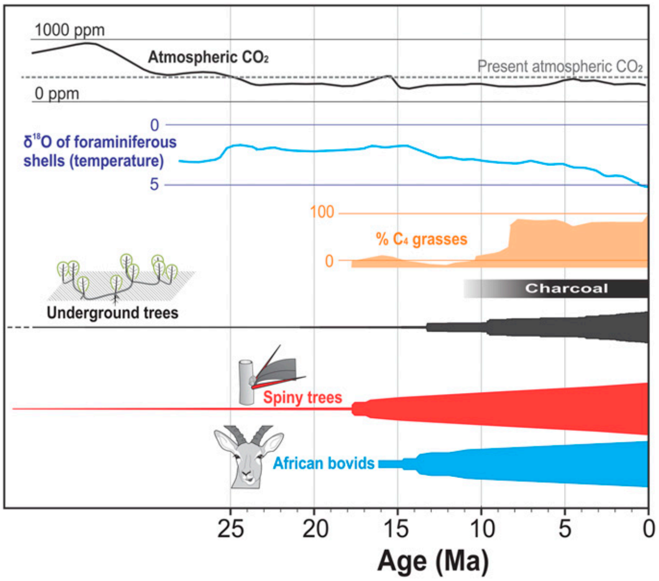

Fig. 5. Potential factors responsible for the rise of savannas. Percentages of C4 grasses reconstructed from $\delta^{13}C$ of tooth enamel of mammalian herbivores (49), atmospheric CO_2 measured from Antarctic ice cores (50), temperature inferred from ^{18}O levels in foraminiferous shells in marine sediments (51), and charcoal abundance from marine sediments (9). For underground trees (12), spiny trees, and African bovids (24), line widths are proportional to the log of lineage numbers.

The structure of the first savannas is intriguing: were they similar to modern savannas but with a C_3 grass layer (1, 4), or were they carved out of ancient thickets as browse pressure increased? How do the distinct histories of browsing on other continents relate to the evolution of structural defenses? Answers to these questions require a better understanding of the interactions between photosynthetic constraints and efficacy of defense and of how structural defenses vary with different modes of feeding.

Materials and Methods

Taxon Sampling and Plant Distribution Data. We sampled a total of 1,852 (of ~2,200) woody plant species in southern Africa from 127 families and 651 genera, including 213 spiny taxa. Species names were extracted from the African plants database (www.ville-ge.ch/cjb/) and cross-checked against The Plant List (www.theplantlist.org/). Species were defined as spinescent if they had hard sharp-pointed structures developed from modified epidermis (prickles), modified stipules (stipular spines), or lignification of the apex of a stem (thorns) (Fig. S1).

Locality records for each species were extracted from the African plants database (www.ville-ge.ch/cjb/) and supplemented with records from the Naturalis Biodiversity Center (www.naturalis.nl/nl/) and the Global Biodiversity Information Facility (www.gbif.org). All records were thoroughly checked, and those with points falling in the sea, inverted latitude/longitude, or duplicate records were removed. Genera with fewer than 10 records were also excluded from the analysis. Species distribution models were constructed using MaxEnt (details are in *SI Materials and Methods*).

Environmental Variables. Environmental variables were derived from WorldClim (44) (mean annual rainfall and temperature), the Food and Agriculture Organization of the United Nations (www.fao.org/home/en/; soil nutrient status "SQ1"), and the Global Fire Emissions Database (45) (annual mean burn percentage for 1997–2009) and downscaled to a 1° × 1° grid resolution. Vegetation classifications were based on major vegetation types and mosaics for Africa by White (46). The original 80-level vegetation mapping units scheme by White (46) was simplified to 15 vegetation types based on the relative predominance of plant growth forms and functional groups (Figs. 1 and 2).

Large mammal herbivore functional types and biomass surfaces were obtained from the work in ref. 23. Herbivore functional types were based on hierarchical cluster analysis of five species-level traits (body mass, diet, gut type, herd size, and water dependence) for 92 African herbivore species. Biomass surfaces were created from spatially explicit species-level historical biomass reconstructions (∼1,000 y ago) produced from models of protected area census data in relation to rainfall, soil, and vegetation.

Phylogenetic Reconstruction and Dating. Phylogenetic reconstruction and dating for trees follow the work in ref. 12 (details are in *SI Materials and Methods*). Phylogenetic reconstruction for bovids is from the work in ref. 24, and it is a dated phylogeny based on the full mitochondrial genome and calibrated with 16 fossils.

Statistical Analyses. We first investigated environmental factors that correlate with the spatial distribution of spinescence to (*i*) identify the group of herbivores most associated spatially with the presence of spiny vegetation, (*ii*) reveal the environmental factors favoring spinescence, and (*iii*) explore the interaction between abiotic environment, spiny species, and herbivory.

Variables from the three datasets—herbivore densities, abiotic factors, and vegetation types—were standardized and analyzed using canonical correspondence analyses (CCAs). Stepwise selection in CCAs (permutation under reduced model) (47) was applied to each dataset separately using a stopping criterion of 0.05. We then used variation partitioning to estimate the unique and joint effects of herbivores, abiotic environment, and vegetation type on the proportion of spiny species. CCAs and partial CCAs were used with three partitions, and the significance of testable fractions was evaluated using permutation tests (9,999 permutations; $\alpha < 0.05$) (47). For each of these analyses, adjusted R^2 values provided unbiased estimates of the variation explained by the fractions (48). Venn diagrams were used to illustrate the results of variation partitioning.

Phylogenetic logistic regression was used to assess the extent to which spines are associated with particular environments and forms of herbivory pressure across plant lineages. Species-specific climate and herbivory scores were obtained by averaging each of four environmental variables (mean annual rainfall, mean annual temperature, soil nutrient status, and percentage burned area) and the biomass of five herbivore functional types (small nonsocial browsers, medium-sized social mixed feeders, large browsers, water-dependent grazers, and nonruminants) across their distribution range. Because of the strong correlations among variables, principal components analysis was performed to obtain orthogonal axes for inclusion in the subsequent regression analysis. Spinescence, coded as spiny = 1 and nonspiny = 0, was then modeled using logistic regression with the first three principal components (87.5% of total variation) (Table S1) fitted as explanatory variables and the nonindependence among residuals informed by the phylogenetic tree (phyloglm using maximum penalized likelihood estimate; R Statistical Software). Model selection was based on Akaike information criterion scores, with only significant predictor variables described. Variable loadings on principle components (Table S2) were used to gauge environmental and herbivory effects on spinescence. All analyses were conducted in the following R packages: FactomineR, vegan, packfor, and phylolm.

Spatial autocorrelation in the residuals accounted for only a very small part of the total variation in both our spatial analyses (Mantel correlation = 0.0015) and phylogenetic logistic regressions (cumulated Mantel correlation always smaller than 0.04) (Table S3); we, therefore, did not fit an explicit description of spatial structure into the models.

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