



Sixty-six million years along the road of mammalian ecomorphological specialization

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The fossil record of the large terrestrial mammals of the North American Cenozoic has previously been quantitatively summarized in six sequential episodes of faunal associations—“evolutionary faunas”—that correspond well with previously proposed qualitative “Chronofaunas.” Here, we investigate the ecological spectrum of these faunas by classifying their major taxonomic components into discrete ecomorphological categories of diet, locomotion, and body size. To specifically address the potential influence of long-term climatic shifts on the ecomorphological composition of these faunas, we analyze via contingency tables and detrended correspondence analyses the frequency distribution of ecomorph types within faunas. We show that each evolutionary fauna has a unique, nonrandom association of ecomorphs, and we identify a long-term trend toward greater ecomorphological specialization over successive faunas during the past 66 My. Major vegetation shifts induced by climatic changes appear to underlie the ecomorphological dynamics of these six temporal associations that summarize Cenozoic North American mammalian evolutionary history.

Cenozoic mammals | Cenozoic climatic change | ecomorphology | evolutionary faunas

Climatic change and the associated vegetational shift influence patterns of faunal dynamics over deep time (1–3). For this reason, understanding the influence of these changes on the biota is fundamental for modeling future biotic responses to anthropogenically induced climatic and environmental change (4, 5).

The fossil record provides a unique opportunity to understand how the biota has responded to climatic fluctuations and vegetation shifts over macroevolutionary timescales (6). Terrestrial mammals are an excellent group to use for investigation of the effects of changes in climate and vegetation on biodiversity, as they are clearly influenced by such factors today (7) and have an excellent fossil record (8, 9), which affords the opportunity of analyzing patterns of ecological change and biodiversity dynamics over deep time (10–12).

A number of paleoclimatic proxies show a general overall trend of cooling and increased aridity through the North American Cenozoic (the past 66 My), punctuated by several warmer and wetter intervals (13–15). This has led to large-scale changes in vegetation over much of the continent consisting of a shift from predominantly tree-covered habitats in the Paleogene to grasslands in the Neogene (16, 17). Studies of large mammalian herbivores (ungulates: hoofed mammals; here including proboscideans) and small ones (Glires: rodents and rabbits) document parallel changes in dietary/locomotor strategies through time (18).

Figueirido et al. (12) showed that the generic diversity of large terrestrial mammals (primarily families of ungulates and carnivores including large >1 kg representatives) of the North American Cenozoic can be quantitatively summarized into six megaassociations of taxa or “evolutionary faunas” (19). These empirical faunas, which emerged as covarying mammalian clades in a *Q*-mode factor analysis, (i) share specific patterns of evolutionary dynamics (i.e., times of origination, maximum diversification, and extinction) that correlate with patterns of global climate change; and (ii) broadly correspond with the classic North American

qualitative mammalian “Chronofaunas” (20). Here, we investigate the ecomorphological spectrum of these six evolutionary faunas by analyzing changing patterns in ecomorph (21) diversity, considering categories of diet, locomotion, and body size. As in our previous study, determinations are based on an analysis at genus level of those taxa that diversified in synchrony rather than on raw data of standing generic diversity per time interval. For a given fauna, this allows the exclusion of those taxa that are asynchronous or that have very low, or more stable, standing diversities across several time intervals. Accordingly, we classified the genera of the most significant families/subfamilies (i.e., those scoring above 1.0 in each of the evolutionary faunas of ref. 12) into different ecomorphs (Table 1 and *SI Appendix*, Table S1 and Fig. S1) related to dietary strategies, locomotor modes, and body size classes (22, 23). These ecomorphological categories follow those in the NOW (<http://www.helsinki.fi/science/now/>) database (24).

The dietary and locomotor categories of the taxa shown in Table 1 can be broadly ordered in increasing level of ecomorphological specialization (i.e., toward more restricted niche breadth) from generalized (presumably more eurytopic) forms to more specialized (i.e., presumably more stenotopic) ecomorphs (e.g., in the case of herbivores, from plant-dominated omnivores with generalized ambulatory locomotion to hypercursorial grazers; *Methods*). As in Figueirido et al. (12), we included only large terrestrial mammals and performed contingency table analyses and detrended correspondence analyses (DCAs) from those ecological determinations that characterized the evolutionary faunas to specifically address the following questions: (i) Does each evolutionary fauna of large terrestrial mammals have a distinctive nonrandom association of ecomorphs? (ii) If so, which ecomorphs characterize each of the faunal associations and which define their differences? (iii) Could these long-term

Significance

The six “evolutionary faunas” of large mammal taxonomic diversity described for the North American Cenozoic have a nonrandom ecomorphological spectrum and show a long-term trend toward greater ecological specialization over the past 66 My. We show here that each successive fauna was characterized by a change toward more specialized ecotypes; these changes were correlated with vegetation shifts caused by major climatic changes, which may have promoted the appearance of new ecological opportunities and morphological innovations.

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Table 1. Analysis of adjusted residuals from contingency tables

	Evolutionary faunas					
	I	II	III	IV	V	VI
Ecomorphs	Pu0-Wa7	To2-Ui3	Wa7-Wh2	Ui3-He2	Wh-BI	He2-RLB
Diet						
Plant-dominated omnivore (Pdo), 50–70% of plant resources	20.462***	8.049***	−5.887	−6.592	−6.174	−5.030
Folivore–frugivore (Ffr), >50% of fruits and <25% of grass	−5.113	8.538***	16.654***	−3.882	−8.183	−6.535
Browser (Brw), <25% of grass and <50% of fruits	−3.236	−7.025	1.725	12.038***	0.851	−4.831
Mixed-feeder (B-g), 25–75% of grass	−5.084	−6.810	−6.239	0.904	6.607***	7.907***
Grazer (Grz), >75% grasses	−4.378	−5.864	−5.373	−6.016	8.156***	10.684***
Animal-dominated omnivore (Ado), 50–70% of animal resources	5.229***	−5.823	−5.335	2.057*	3.616***	0.470
Meat (Me), generalized carnivore, 50–70% of meat	−1.823	6.731***	3.641***	−2.983	−1.715	−3.487
[†] Meat–bone (Meb), hypercarnivore (>70% of flesh) feeding on meat and bone marrow	−2.735	0.081	−0.825	2.601***	3.554***	−3.459
[†] Meat–insect (Mei), invertebrates and small animal prey	−1.337	−1.790	−1.640	−1.837	−2.139	8.075***
Meat-only (Meo), hypercarnivore (>70% of flesh) feeding only on meat	−3.282	1.664	−0.961	2.867***	−4.735	3.961***
Locomotion						
Generalized quadrupeds (Gqu), Generalized quadrupedal	19.628***	11.357***	−3.409	−12.376	−6.483	−3.884
Subcursorial (Scu), Running forms (low degree)	−8.682	3.266**	13.192***	10.751***	−7.369	−10.614
Cursorial (Cur), Running forms (moderate degree)	−5.746	−7.696	−7.068	10.241***	8.411***	−0.730
[†] Hypercursorial (Hyp), Running forms (high degree)	−6.646	−8.903	−8.176	−6.298	10.151***	15.865***
[†] Graviportal (Gra), Very large sized ungulates	−2.288	−3.065	7.422***	−2.735	−3.662	4.445***
Body size						
Body size class 1 (Bs1), <1 kg	19.771***	5.967***	−3.614	−6.127	−6.952	−4.207
Body size class 2 (Bs2), 1–5 kg	0.922	5.975***	2.064*	−1.343	−2.770	−3.929
Body size class 3 (Bs3), 6–19 kg	−4.569	3.922***	3.340***	0.098	−0.006	−3.251
Body size class 4 (Bs4), 20–89 kg	−3.397	−4.889	−2.622	3.279*	5.151***	0.956
Body size class 5 (Bs5), 90–199 kg	−4.675	−4.165	−1.521	3.648***	2.567**	2.609**
Body size class 6 (Bs6), 200–299 kg	−2.809	−5.387	−1.337	−1.445	5.091***	4.384***
Body size class 7 (Bs7), 300–949 kg	−2.155	−2.893	3.216**	0.396	−2.687	3.911***
Body size class 8 (Bs8), 950–3,000 kg	−2.045	−2.745	3.179**	2.041*	−3.273	2.775***
Body size class 9 (Bs9), >3,000 kg	−1.293	−1.736	−1.591	−1.774	−2.070	7.819***

Adjusted residuals from the three contingency tables computed for diversity of genera weighted by species classified within each ecomorphological category among the six evolutionary faunas. Separate contingency tables were computed for each category (trophic preferences, locomotion modes, and body size classes). The level of significance (two-tailed *t* test; **P* < 0.05; ***P* < 0.01, ****P* < 0.001) is also shown. The morphological correlates of each ecological category are defined in *SI Appendix, Table S2*. Abbreviations: BI, Blancan; He, Hemingfordian; Pu, Puercan; To, Torrejonian; Ui, Uintan; Wa, Wasatchian; Wh, Whitneyan. The body size category of <1 kg includes the smallest members of the large mammal orders analyzed (some miacoids and condylarths, small musteloids, etc.). Small mammals (e.g., glires, insectivores, and noneutherian taxa) are not analyzed here.

[†]Denotes specialization equality (*SI Appendix*).

ecological changes be related to major Cenozoic climatic shifts and resulting changes in vegetation? In addition, given that Glires occupy the same habitat and use the same food resources as ungulates, we discuss whether both groups respond to these changes in a synchronized fashion.

Results and Discussion

Fig. 1*A* shows the distribution of ecomorphs based on generic diversity weighted by species of each dietary type, locomotor mode, and body size class. Table 1 shows the three contingency tables performed to characterize the evolutionary faunas. The cumulative values of the χ^2 tests were significant for the distribution of genera among dietary types, locomotor modes, and body size classes (diet: $\chi^2 = 1,678.5$; *df* = 50; *P* < 0.001; locomotion: $\chi^2 = 346.96$; *df* = 20; *P* < 0.001; body size: $\chi^2 = 811.72$; *df* = 40; *P* < 0.001), which indicates a nonrandom distribution of genera among ecomorphological types and evolutionary faunas. The analysis of the adjusted residuals of the contingency tables (i.e., standardized differences between the observed and expected frequencies of each ecomorph in each fauna; *Methods*) indicates that a distinct association of ecomorphs characterizes the ecological spectrum of each evolutionary fauna (Table 1). Ordination plots derived from DCAs confirm overall the unique

ecological spectrum of each evolutionary fauna, as those ecomorphs that are particularly abundant in a given evolutionary fauna plot in close proximity to that fauna (Fig. 1*B*).

Ecomorphological Spectrum of Cenozoic Mammalian Faunas. After the Cretaceous–Paleogene (K–Pg) mass extinction event, there was an extremely rapid diversification of placental mammals (25, 26) that heralded the Paleocene fauna (evolutionary fauna [EV] I; time range, Puercan0 to Wasatchian7). This fauna peaked in generic diversity at the Paleocene–Eocene thermal maximum (PETM) (~61.5 Ma) (12), and the predominant vegetation in North America was paratropical forest (27). The Paleocene fauna of large, terrestrial mammals was mainly dominated by omnivorous archaic types of mammals such as “condylarths” (a paraphyletic or polyphyletic assemblage of archaic ungulates) feeding on both animal and plant resources; these mammals were in general of small body size (in comparison with extant carnivorans and ungulates) and had a generalized quadrupedal mode of locomotion (Table 1 and Fig. 1*A*). The combination of dietary and locomotion ecomorphs indicates that this evolutionary fauna comprised a unique assemblage of large mammals in feeding types but not in locomotion modes (Fig. 1*B*).

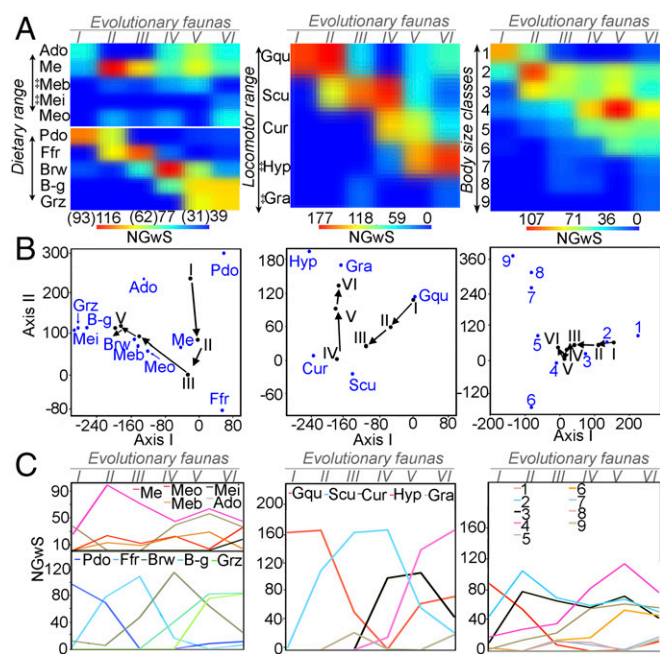


Fig. 1. Ecomorphological analysis of the six evolutionary faunas of North American mammals. From *Left to Right*: dietary, locomotion, and body size categories. (A) Matrix plots performed from the number of genera weighted by species (NGWS) per ecological category distributed along the six evolutionary faunas. In the dietary categories, herbivores are plotted separately from carnivores for clarity. The ecomorph categories are ordered from *Top to Bottom* in increasing level of specialization, and so the predominance of warm colors (i.e., higher NGWS values) in the main diagonal stripe indicates a trend toward greater ecological specialization along the successive faunas. †Denotes specialization equality (*SI Appendix*). The black arrows indicate the path through the successive evolutionary faunas. (B) Bivariate plots depicted from the scores of the first two axes obtained from a DCA computed from the weighted generic diversity of ecomorphs within each evolutionary fauna. (C) Bivariate plots for number of species per genus (NGWS) within each ecomorph (x axis) against faunas (y axis). See Table 1 for abbreviations.

The late Paleocene warming that peaked at the PETM coincided with the transition from the Paleocene fauna (EV-I) to the early–middle Eocene fauna (EV-II; time range, Torrejonian2 to Uintan3), reaching its maximum generic diversity during the early Eocene climatic optimum (EECO) at ~52–50 Ma (12). Its distinctive elements were generalized carnivores as well as folivores–frugivores (i.e., herbivores specializing on high-quality, low-fiber plant parts). Plant-dominated omnivores and more specialized carnivores (e.g., hyaenodontid creodonts) were also significant members of it (Table 1 and Fig. 1A). Change in dietary habits to include more leaves (i.e., greater folivory) may relate to changes in vegetation: Open canopy woodlands were present (28), which may have resulted in a greater diversity of undergrowth herbage for terrestrial forms as opposed to the more closed-canopy forests of the Paleocene. Compared with the Paleocene fauna, members of this fauna were also more specialized in locomotion strategies, as subcursorial taxa (i.e., running forms but of a low degree) were significant components of this fauna (Table 1 and Fig. 1A). This probably relates to the first appearance of basal artiodactyls and perissodactyls at this time. However, the close position of the early–middle Eocene fauna to the Paleocene one in the locomotion DCA space, but not in the dietary DCA space (Fig. 1B), reveals a decoupling in dietary and locomotor specializations among the members of this fauna. Moreover, these forms were generally larger than those of the Paleocene fauna, with most taxa being ~5 kg or more (Table 1 and Fig. 1B). Note that, while this fauna contained numerous

new immigrant mammals (*SI Appendix, Table S1*), the ecomorphological spectrum shows relatively minor changes from the Paleocene to the early Eocene. Rodents, which first appeared in the latest Paleocene, also showed a rapid increase in taxonomic diversity in the early Eocene (18).

A 17-My period of cooling after the EECO correlated with the eventual demise of the early–middle Eocene fauna (EV-II) and the subsequent rise of the middle–late Eocene one (EV-III; peak, ~44 Ma; time range, Wasatchian7 to Whitneyan2) (12). This faunal replacement is characterized by a further increase of folivore–frugivore herbivores as well as by the continuation of generalized carnivores, and the first appearance of true browsers (e.g., basal rhinos and tapiroids), but the latter are not a significant component of this fauna. A particular difference in this fauna from the preceding ones is the emergence of more specialized types of locomotion, with the appearance of graviportal forms (brontotheres) and a radiation of subcursorial ungulates and carnivores that first appeared in EV-II. The mammals in this fauna also show a bimodal size range, with an emphasis on both small (size classes 2–3, ~1–19 kg) and large to very large (size classes 7–8, ~300–3,000 kg) forms (Table 1 and Fig. 1B). There was a significant vegetation shift in the later Eocene to floras indicative of cooler and drier conditions, with significant seasonality (27, 29), and the decline of arboreal mammals such as primates (18). These ecomorphological changes reflect a greater dependence on leaves in the diet (deciduous leaves are less structurally protected, hence more palatable, in seasonal habitats) and a locomotor spectrum indicative of more open habitats. Rodent taxonomic diversity fluctuated but remained high through the late Eocene, and lagomorphs made their first appearance in the middle Eocene at ~42.5 Ma (18, 30).

Although the middle Eocene climatic optimum (MECO) (~41.5 Ma) (27) correlates with the transition between the middle–late Eocene (EV-III) and the Oligocene (EV-IV; peak, 30–32 Ma; time range, Uintan3 to Hemphillian2) faunas, the rise of the latter correlates with an immigration event from Asia (12, 31). The Oligocene fauna is characterized by the appearance of a number of new ecomorphs, including specialized hypercarnivores and the radiation of browsing herbivores. The hypercarnivores eating meat alone (e.g., nimravids [false saber-tooths]) first appear during this fauna, and those hypercarnivores eating meat and bone (some large hesperocyonine canids [e.g., *Sunkahetanka*, *Enhydroyon*]) also make a first appearance in this fauna. However, more specialized bone-crackers do not diversify until the Miocene fauna, where they become a more significant component. In fact, the forms of the Oligocene fauna show less advanced craniodental features indicative of bone crushing than those present in large borophagine canids from the Miocene fauna (e.g., *Aelurodon*, *Borophagus*, and *Epicyon*). The herbivores of the Oligocene fauna comprise mainly browsers, including forms with a cursorial type of locomotion (e.g., equids and basal ruminants), and a few mixed feeders (leptauchinine oreodonts, but these are not a significant component of the fauna). Subcursorial forms continue, and animal-dominated omnivores make a reappearance (e.g., among basal borophagine canids). The bimodal size range observed in the preceding fauna is maintained, with an overrepresentation of forms in size classes 4–5 (~20–199 kg) and 8 (~950–3,000 kg) (Table 1 and Fig. 1A and B). The substantial change in the ecomorphological spectrum of the Oligocene fauna relative to both preceding Eocene faunas emphasize the significance of the global cooling of the Eocene/Oligocene transition (32, 33).

With regards to the Glires, their taxonomic diversity rises dramatically in the middle and late Oligocene, reaching a maximum in species richness not seen again until the Pliocene; this increase is due to the diversification of forms with hypsodont and hypselodont (i.e., ever-growing, unrooted) teeth, which surpass the diversity of brachyodont taxa (18, 34).

The temperature rises at the beginning of the late Oligocene correlates with the rise of the Miocene fauna (EV-V; time range: Whitneyan2 to late Blancan) (12). This warming peaked with the Middle Miocene Climatic Optimum (MMCO) (~17–14 Ma), which coincided with both the demise of the Oligocene fauna (EV-IV) and the peak of the Miocene one (EV-V) at ~16.5 Ma. Animal-dominated omnivores (e.g., procyonids and some mustelids), cursorial mixed-feeding herbivores (e.g., ruminants and camelids), and predominantly cursorial meat–bone eaters (e.g., borophagines) characterize the ecological spectrum of the Miocene fauna. Furthermore, hypercursorial grazing herbivores (equine equids) now make their first appearance (Table 1 and Fig. 1 *A* and *B*). The Miocene fauna shows an increase of species of medium-to-large body sizes (size classes 4–6, ~20–300 kg) (Fig. 1*A* and Table 1). These ecomorphological changes accompany major climate and vegetation changes, including the spread of savanna grasslands at the beginning of the Miocene (17).

Strikingly, there is a significant absence of specialized hypercarnivores in the Miocene fauna, which probably reflects the absence of “cat-like” predators (35). However, despite this absence, the Miocene fauna is characterized by the diversification of specialized bone-crushing carnivores (borophagines and some mustelids). Borophagines reached their maximum taxonomic diversity in the Miocene and were probably exploiting both hypercarnivorous dietary strategies (i.e., meat-only and meat–bone) (2, 36, 37).

With regards to the Glires: following a late Oligocene decline, rodent taxonomic diversity rises in the middle Miocene but declines again in the late Miocene. In contrast, the taxonomic diversity of lagomorphs is low in the earlier Miocene but rises in the late Miocene (18).

Finally, the Plio-Pleistocene fauna (EV-VI; time range: Hemphillian2 to Rancholabrean) commences at ~16.5 Ma (i.e., coincident with the peak of the Miocene fauna) and reaches a maximum generic diversity in the late Pliocene (~3.5 Ma). Its inception broadly correlates with a late early Miocene dispersal event from Asia (10). The immigrant components of this fauna (Felidae, Procyonidae, Mustelinae, and Gomphotheriidae) may have also played a major role in the decline of the Miocene fauna (12), but changes in climate and vegetation at this time include significant cooling and drying following the MMCO, and change from the early Miocene woodland savannas to cool-temperate grasslands and woodlands (27, 38).

This new Plio-Pleistocene stock of taxa is mainly characterized by the presence of specialized hypercarnivores (represented by large felids, including saber-tooths) as well as by the continuation of grazing and mixed-feeding herbivores with hypercursorial (equids, ruminants, camelids) and graviportal (proboscideans) modes of locomotion. Many taxa are large (size classes 5–7, 90–949 kg) and there are also a number of large megaherbivores among the proboscideans in size class 9 (>3,000 kg) (Table 1 and Fig. 1 *A* and *B*). The absence of meat–bone carnivores as a significant component of this fauna relates to the decline and subsequent extinction of borophagines (35, 36). Interestingly, the only bone-crackers were the slender-limbed, wolf-like hyena *Chasmaporthetes*, a Pliocene Old World immigrant that did not diversify in North America (39), and the borophagine *Borophagus diversidens*. Meat–insect-eating carnivores, which are a significant component of this fauna and unique to it, represent an entirely new dietary specialization due to the radiation of mustelids. This could relate to the spread of prairies in North America, which resulted in an increase of the biomass available of insects (40).

The taxonomic diversity of small mammals rose substantially in the latest Miocene and early Pliocene, in part relating to immigration, reaching a taxonomic diversity peak that was surpassed only by the present day (possibly explained by the Pull of the Recent).

Sixty-Six Million Years of Ecomorphological Specialization. The finding of significant positive residuals for a number of dietary types, locomotor modes and body size classes through the six evolutionary faunas (Table 1) indicates an increase in ecological specialization during the past 66 My of mammalian faunas in North America, reflecting changes in climate and vegetation. This is particularly evident among herbivorous forms: While the Paleocene taxa are largely generalized omnivores, and folivores–frugivores diversify during the Eocene, browsing taxa radiate during the Oligocene, and mixed-feeders and grazers are abundant during the Miocene and Plio-Pleistocene (Table 1, Fig. 1*C*, and *SI Appendix*, Fig. *S1*). However, the increase in dietary specialization among carnivores is not as clear as with the herbivores: The type of vegetation available changes with climatic conditions, but flesh remains unchanged as a food resource (although hunting style may vary).

Omnivores with animal-dominated diets first radiate during the Paleocene, disappear during the Eocene, radiate again during the Oligocene and Miocene, and finally decline in the Plio-Pleistocene (Table 1 and Fig. 1*C*). Generalized carnivores radiate during the Eocene, but the carnivores of the Oligocene and Miocene are more specialized hypercarnivores, including meat–bone consumers (Table 1 and Fig. 1*C*). The initial Oligocene radiation of hypercarnivores coincides with the first radiation of browsing herbivores. Although specialized grazers first radiate within the Miocene fauna, hypercarnivores are not a significant component of this fauna, probably reflecting the “cat-gap” (35). Finally, the Plio-Pleistocene fauna shows a second wave of diversification of hypercarnivores feeding exclusively on meat (i.e., not including bone), which takes place in parallel with a major diversification of grazing and mixing-feeding herbivores, the decline of bone-cracking carnivores, and the appearance of carnivores including insects in their diet (Table 1 and Fig. 1*C*).

The weighted generic diversity of locomotor modes also shows a greater specialization over the successive faunas: Generalized quadrupeds characterize the Paleocene fauna and the first Eocene fauna; subcursorial mammals increase their abundance from the second Eocene fauna to the Oligocene one; cursorial and hypercursorial forms dominate the Miocene fauna; and graviportal mammals appear as significant components of the Plio-Pleistocene fauna, in which hypercursorial forms show a second pulse of diversification (Table 1 and Fig. 1*C*). The only exception is the presence of graviportal forms in the middle-late Eocene fauna, which reflects the diversification (and subsequent extinction) of brontotheriids.

Previous authors (e.g., ref. 41) have suggested that ecological diversification to fill ecological niches coupled with environmental factors (e.g., temperature or land area) were the primary explanatory factors of evolution of large body sizes in terrestrial mammals. Our ordination of body size categories in mammalian faunal dynamics over deep time suggests a body size increase through the successive faunas (Fig. 1 *A* and *C*). However, this pattern is not as clear as for diet and locomotion. The Paleocene fauna and the first Eocene fauna are characterized by the diversification of forms of small body size (size classes 1–2), although a few larger taxa (e.g., pantodonts, up to size class 7) are also present. Small- to medium-sized mammals (size classes 3–4) increase in abundance in the middle-late Eocene and Oligocene faunas, while somewhat larger mammals (size classes 4–6) are significant components of the Miocene and Plio-Pleistocene faunas. Very large mammals (size classes 7–9) first appear in the middle-late Eocene fauna (brontotheres) (Table 1 and Fig. 1 *A* and *C*), but these size classes are occupied by different lineages in later faunas (e.g., rhinos or proboscideans).

Faunal Dynamics of Large and Small Mammals. The ecomorphological dynamics of large and small mammals respond to climatic changes and vegetation shifts (8, 10, 18). However, while small mammals respond to these changes relatively tightly, larger ungulates

may show a delayed response, up to several million years. A classic example is the response to the appearance of grass-dominated habitats in the early Miocene. Rodents and rabbits evidenced rapid dietary change (18), while although horses were apparently exploiting these habitats during the early Miocene by 23 Ma (1), the major morphological shift for eating grasses—hypsodont (high-crowned) cheek teeth—did not appear in the equid lineage until around 5 My later (42, 43). Similarly, while an increase in cursorial (running-adapted) ungulates took place from the late Oligocene through the Miocene, concurrent with the spread of open habitats, most large predators remained ambush specialists until the late Miocene and through to the Pleistocene, when true pursuit predators appeared and diversified (2, 36, 37, 44).

The decoupling of the appearance of grassland habitats (1, 17, 34, 44, 45) and the evolution of craniodental adaptations for a grazing diet in ungulates is likely a consequence of the ability of larger animals to range widely, due to their relatively lower costs of locomotion, and hence to avoid areas of less favorable dietary items. In contrast, small mammals (which show parallel changes in ecomorphological types through the North American Cenozoic) have smaller home ranges, a restricted ability to disperse and shorter generation times (which would result in higher evolutionary rates), which renders them more sensitive to local changes in climate and vegetation (18).

Conclusions. Our results demonstrate that the six evolutionary faunas of North American mammals (12) are ecomorphologically as well as taxonomically coherent. The statistical analysis by contingency tables of raw data on weighted generic diversity of ecomorphs among these faunas indicates that dietary types, locomotion modes, and body size classes are not randomly distributed. Instead, each fauna had its own unique combination of ecomorphs that defined its ecological spectrum. This indicates that the analysis on those taxa that experienced coupled diversification processes in the evolutionary faunas could help to amplify the biological signal contained in the fossil record.

Our analysis reveals a greater ecological specialization of large, terrestrial mammals over the successive evolutionary faunas, with an increase in the relative frequency of derived feeding types and locomotion modes, in parallel to an overall increase in the range of body sizes through time. The long-term climatic and vegetation shifts in the North American ecosystem are well documented and reflect global higher-latitude climatic changes over the Cenozoic. The tropical-like forests of the Paleocene and early Eocene were replaced, following higher-latitude cooling, by seasonal woodlands and scrublands in the late Eocene and Oligocene. This was followed by the diversification of savanna-like grasslands in the warming of the Miocene that turned to prairie in the cooling and drying of the Plio-Pleistocene. The changing dominant habitats correlated with (and probably drove) the evolutionary changes in the animals inhabiting them. Increasingly specialized forms (hypercarnivores, grazers, cursors) took advantage of new ecological opportunities, and less specialized ecomorphological types (omnivores, generalized quadrupeds) became less common. Some of the new ecomorphological types represent immigrant taxa [e.g., pecoran (horned) ruminants, proboscideans], but similar changes also occurred among the endemic members of the fauna (e.g., equids, camelids, canids). We show here that these ecomorphological changes over time took place in a synchronized fashion, as was also seen with the taxonomic changes in distinctive assemblages of large mammals (i.e., evolutionary faunas; ref. 12).

Finally, the comparison with the faunal assemblages of small mammals (18) shows parallel—but uncoupled—changes in ecomorphological types through the North American Cenozoic. For this reason, future studies should quantitatively compare the evolutionary dynamics of both large and small mammals for modeling the response of mammalian faunas to climatic change and vegetation shift.

Materials and Methods

To analyze the dietary, locomotion, and body size structure of each mammalian evolutionary fauna reported in ref. 12, the genera of each family/subfamily taxonomic unit present in these temporal associations were classified according to the ecological categories described in Table 1, information now available in the NOW database (24). We use the terms “generalized” and “specialized” in an ecological sense, as equivalents of eurytopic and stenotopic, respectively. In contrast, we do not follow the common practice of equating specialized ecology with derived (apomorphic) morphology, and generalized with ancestral (plesiomorphic), as apomorphic characters may well enable ecological generalization (e.g., ursids show a highly derived morphology compared with their ancestors and have a generalized diet). Following ref. 46, we considered an ecomorph as specialized if its range of tolerance for the resources consumed is included within that of the generalist (see *SI Appendix* for details). All species within a genus were assigned to the same ecomorphological categories, and the analyses were based on genera weighted by the number of species within each genus.

We analyzed 1,231 large mammal species distributed over 436 genera (*Dataset S1*). This study is based on the same dataset as in Figueirido et al. (12) and at the same taxonomic level. We here classify the genera of those families/subfamilies that defined the evolutionary faunas into discrete ecomorphological categories to determine whether these faunas can be characterized not only in terms of taxonomic composition, but also in terms of the ecomorphology of the contained genera. In Figueirido et al. (12), each evolutionary fauna was named according to the time period where it peaked in diversity rather than its full temporal range. The time span of each fauna is given in Table 1.

The weighted generic diversity (i.e., genera weighted by species) of each ecomorphological category in each of the evolutionary faunas was tested statistically using three contingency tables, one for each category (i.e., diet, locomotion, and body size classes). Each contingency table has r rows (ecomorphs) and c columns (evolutionary faunas). The statistic for testing against independence between ecomorphs and faunas is as follows: $\chi^2 = \sum_j = 1^c \sum_i = 1^r (O_{ij} - E_{ij})^2 / E_{ij}$, where O_{ij} is the observed frequency of ecomorph i in fauna j for the ij th cell (i.e., n_{ij}) and E_{ij} represents the expected frequency for this cell under the null hypothesis of independence (i.e., a random distribution of ecomorphs among the faunas). The latter is computed as follows: $E_{ij} = (\sum_i = 1^r n_i \cdot \sum_j = 1^c n_j) / (\sum_i = 1^r \sum_j = 1^c n_{ij})$, where n_i and n_j are the total number of cases that show the i th and j th attributes [i.e., partial sums for rows (ecomorphs) and columns (faunas) in the table, respectively]. When the null hypothesis holds, χ^2 is approximately distributed as a χ^2 variable with $(r - 1) \cdot (c - 1)$ degrees of freedom.

The individual cells of the contingency tables were also analyzed independently using the method of adjusted residuals (47). This allows the determination of which ecomorphological types (rows) are significantly overrepresented in each evolutionary fauna (columns). Let $e_{ij} = (O_{ij} - E_{ij}) / E_{ij}^{1/2}$. The mean of this variable equals zero and its variance is $v_{ij} = (1 - n_i/n) \cdot (1 - n_j/n)$, where n is the total sum of ecomorphs in the faunas. The adjusted residuals are $d_{ij} = e_{ij} / v_{ij}^{1/2}$, and they result from standardization (i.e., z-score normalization) of e_{ij} values. Adjusted residuals are approximately normally distributed $[N(0,1)]$ when there is no association between the rows and columns of the contingency table. However, a situation of dependency generates residuals that are higher than the standard normal deviates for a specific level of confidence (e.g., $P < 0.05$). For this reason, when the absolute value of the adjusted residual (d_{ij}) for a given cell is higher than the standard normal deviate (e.g., 1.96 for $P = 0.05$), the null hypothesis of independence is rejected for this cell (a positive value indicates an overrepresentation of genera of the i th ecological category in the j th evolutionary fauna compared with the expectations from a random distribution of ecomorphs among faunas, while a negative one points to an underrepresentation). The distribution of taxa among ecomorphs and faunas was analyzed performing three DCAs, one for each of the three ecomorphological categories. This allowed the ordination of the faunas and ecomorphs in the same ecospace (Fig. 1B). DCA was preferred over other ordination methods because when applied to gradient data it avoids the “arc” and “edge” effects—i.e., a smaller variance of the scores at the beginning and the end of the gradient than at the middle, with the points depicting a horseshoe-shaped curve rather than a straight line (see below). Our main goal was to reveal the ecomorphological structure underlying the evolutionary faunas, as ecomorphological types should be placed close to the faunas that they characterize (48, 49). Furthermore, those faunas with a similar composition of ecomorphs should be close to each other, as should ecomorphological types with similar distributions across the faunas. This is only possible if the structure of the dataset is such that there is a different ecomorphological signature underlying each of the evolutionary faunas. We first performed a correspondence analysis, but as we obtained

a clear “arch artefact” (49), we finally applied DCA to the data to avoid this problem (49). We also applied a DCA algorithm with the modifications suggested by ref. 50 to avoid ordination instability, using PAST, version 3.13 (51).

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