PALEONTOLOGY

A 10-million-year biogeographic holding pen primed the Great American Biotic Interchange

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The movement of taxa between North and South America across the Panamanian land bridge, known as the Great American Biotic Interchange (GABI), established modern-day mammal assemblages in the Americas, heralding the decisive end of what has been called the "splendid isolation" of the South American fauna. Lacking substantive low-latitude records, previous work on this pattern relied on South American and temperate North American fossils to conclude that pulsed interchanges began about 2.8 million years ago (mya). Based on new low-latitude fossil data from México, we found evidence for a prolonged, triphasic interchange. Phase 1 consisted of the initial accumulation of northern higher-latitude mammals in a Mexican "holding pen" beginning 10 mya. Dispersal began in phase 2, with a strong north-south gradient and increasing biogeographic connections (7 to 3 mya). The final phase consisted of the already well-described taxonomic pulses (from ~2.8 mya).

The Great American Biotic Interchange (GABI) transformed terrestrial and marine ecosystems through range expansions driven by changing land masses and sea levels, further leading to subsequent cladogenesis (*1*–*5*). A major wave of overland dispersals and concurrent divergence of marine environments from ~2.8 million years ago (mya) on has been well established by fossil and molecular evidence in birds (*6*), mammals and plants (*4*), marine invertebrates (*7*), marine vertebrates (*8*), and marine microorganisms and corresponding geochemical signatures (*9*). Further, the Plio-Pleistocene Ice Age glacial-interglacial intervals align with four mammal dispersal pulses between 2.8 mya and the end of the Pleistocene (~0.125 mya) (*4*). These findings suggest that the presence of environmental shifts associated with sea-level fluctuations and climate change exerted additional influence over dispersals after persistent terrestrial corridors were established. Among the most profound outcomes of this continental biotic and topographic reorganization is that more than half of living mammal genera in terrestrial South America (of which there are ~217) can be traced to North American founders, compared with only four living North American genera that have South American ancestry (*5*, *10*–*13*).

In contrast to the well-known Plio-Pleistocene biotic interchange events documented in the fossil record, the tempo and mode of terrestrial mammal movements in the ≥10 million years leading up to the interchange pulses are still debated. Although the prevailing paradigm suggests a major wave of bidirectional mammal dispersals starting at ~2.8 mya (*1*, *2*, *4*, *11*), evidence for a more substantial pre-GABI interchange is emerging (*12*, *14*–*17*). The evidence underscoring such dispersals principally hinges on isolated examples of northward-dispersing mammal taxa from South America in low-latitude North America (modern-day México and southern US) for a time before appearing in temperate latitude regions (*4*, *18*) or from migration rate estimates from molecular data (*15*). An implied corollary of this hypothesis is that temperate North American taxa dispersing southward should similarly be found occurring in low-latitude North America earlier than they first appeared in South America. An often noted case of early southward dispersal involves the explosive diversification of sigmodontine rodents in South America. Their fossil record appears during the late Miocene to early Pliocene interval (~7 to 4 mya), yet biogeographic models based on molecular data suggest an earlier diversification between 7.6 and 12.4 mya (*19*). Beyond these kinds of one-off examples, the general validity of connections before the main interchange events has not been evaluated quantitatively using the fossil record. This is despite the potential for such analyses to resolve key controversies in the debate about the timing and nature of early dispersal corridors preceding and leading up to the main interchange pulses that began ~2.8 mya.

The expected paleontological evidence for a prolonged holding pen, a geographic region where organisms temporarily pause or slow down their geographic dispersal, during the interchange entails a latitudinal lag in dispersing taxa as they crossed into and through low-latitude North America. Paradoxically, the quantification of such a holding pen pattern has long been challenged by the highly biodiverse modern-day neotropical environment of low-latitude North America and Central America. Preservation potential for vertebrate fossils in those lushly vegetated regions can be much lower than in arid areas at more temperate latitudes (*20*, *21*). This preservation bias is further compounded by the substantially smaller geographic area covered by modern-day low-latitude North America compared with temperate and higher-latitude regions (e.g., 9.8×10^6 km^2 for the US versus 1.9×10^6 km^2 for México). The resulting asymmetry in the quantity and quality of fossil records across latitudinal gradients severely limits quantitative analyses of past dispersal and biogeographic dynamics. For example, there are 16,085 occurrence entries for mammals identified from the Neogene US in the Paleobiology Database, compared with 640 occurrences from México (The Paleobiology Database, https://paleobiodb.org/#/, accessed 1 October 2025). The records of known Neogene faunas in Central America are even more limited (*21*, *22*). These data asymmetries potentially propagate biased patterns and conclusions and highlight a conspicuous gap in low-latitude fossil records. México is the best candidate for addressing such a data imbalance because the country represents the largest low-latitude North American region with widespread, nearly continuous fossiliferous Mio-Pliocene sedimentary sequences (*23*–*25*). However, research efforts on fossil mammals in México have lagged behind those in the US and Canada in part because of resource and political challenges (*26*, *27*).

Here, we hypothesized that the presence of a prolonged Mexican holding pen (*4*, *11*, *18*) held southward-dispersing mammals for a time before their cross-continental dispersal and that this phenomenon should manifest in (i) diversification within the holding pen region representing the arrival of new taxa temporally antecedent to (ii) a latitudinal lag in biogeographic range expansion in the direction of subsequent dispersal. To test this holding pen hypothesis, we developed a comprehensive dataset of fossil mammals for México, improving the available Neogene records for that country by twofold, from 919 to 1724 unique genus-locality records. We integrated this new dataset with recently updated South American (1920 unique records) and temperate North American (2296 unique records) data and analyzed the combined fossil mammal records from 12 mya to the present.

We tested the hypothesis of an early holding pen by quantifying the timing of biogeographic range expansion using genus-level connectivity

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patterns (number of shared genera per time interval) and net diversification rates (net origination/net extinction of genera) using Bayesian diversity through time analysis. Taxonomic diversity calculations were coupled with occurrence age resampling to account for biochronological uncertainty. A prolonged Mexican holding pen was present by 10 mya, and it played a pivotal role in driving mammal range expansion patterns across the Americas. Our results support an extended, triphasic model of the GABI in which a holding pen preceded a staggered pattern of north then south biogeographic range expansion, and the already well-known dispersal pulses then followed. Both the presence of a sustained holding pen in México and distinctive pre-GABI phases contradict the hypothesis that the Panamanian land bridge formed in the middle Miocene 15 to 13 mya (*16*).

A late Miocene origin of the Mexican holding pen

Diversity through time analyses identified a net diversification peak in México from 10 mya driven by the arrival of mammals that originated in higher-latitude North America (Figs. 1, A and G, and 2). This peak was observed consistently in both raw rate calculations (figs. S3 and S4) and time-variable Poisson process estimations of diversification rates under a Bayesian framework incorporating replicates of resampled taxon occurrence ages (Fig. 2, fig. S6, and supplementary text). Additionally, a nearly contemporaneous diversification peak is observed in South America, but one that is driven by taxa endemic to the southern continent (Fig. 1 and figs. S7 and S8). The Mexican diversification peak is followed by steadily increasing biogeographic connectivity across latitudes in North America, but not South America, until 7 mya. This distinctive phase (Fig. 1, table S1, and see figs. S1 and S2 for a masking of this effect when Mexican data are not disaggregated) indicates continuous range expansion of North American taxa across temperate and low latitudes and provides evidence for interpreting the first appearance of the holding pen at ~10 mya.

A 10-mya origin of the holding pen is at least twice as old as that proposed by Flynn *et al.* (*18*), who documented the arrival of north-bound South American taxa in México at 4.8 mya. The evidence for a much older, late Miocene holding pen centers not only on the accumulation of southbound higher-latitude taxa in low-latitude North America, but additionally includes a latitudinal lag in biogeographic connectivity to South America (Fig. 1G and see below). Rare but important fossil mammal records in Central America indicate that the maximal extent of the holding pen may extend as far as Panama given the absence of South American taxa in late Miocene (9 to 7 mya) faunas in El Salvador, Honduras, and Panama (*21, 22*). The fact that these species had already successfully crossed Nearctic and Neotropical biogeographic regions in North and Central America (*28*) suggests minimal or no ecological barriers. Instead, we suggest that no viable land bridge existed for most mammals between the Americas during the time period from 10 to 7 mya. The exception is the appearance of two sloths, *Pliometanastes* and *Thinobadistes*, which may have crossed under rare, "sweepstakes" circumstances (*1*). This interpretation challenges the middle Miocene timeline for Central American Seaway closure and Isthmus of Panama formation proposed by Montes *et al.* (*16*). Eventually southward-dispersing carnivorans, ungulates, and rodents are well represented among holding pen taxa (Fig. 3B), underscoring how the accumulated taxa represent the same clades that subsequently resumed their southward expansion to South America during the conventional interchange pulses. Furthermore, although late Miocene holding pen mammals were relatively evenly sampled from all available source taxa to the north, they tended to be larger-bodied relative to others in their respective clades (figs. S10 and S11 and tables S2 to S4). The absence of faunal-scale crossings (simultaneous dispersal of multiple species in the same ecological assemblage) between continents supports the hypothesis that the type of corridors present during the main interchange pulses were absent 10 to 7 mya.

The fossil record of the earliest northward-bound mammals, sloths, ~9 mya (*29*) represents an exception to the otherwise well-defined group of mammals within the holding pen region. Sloths are underrepresented

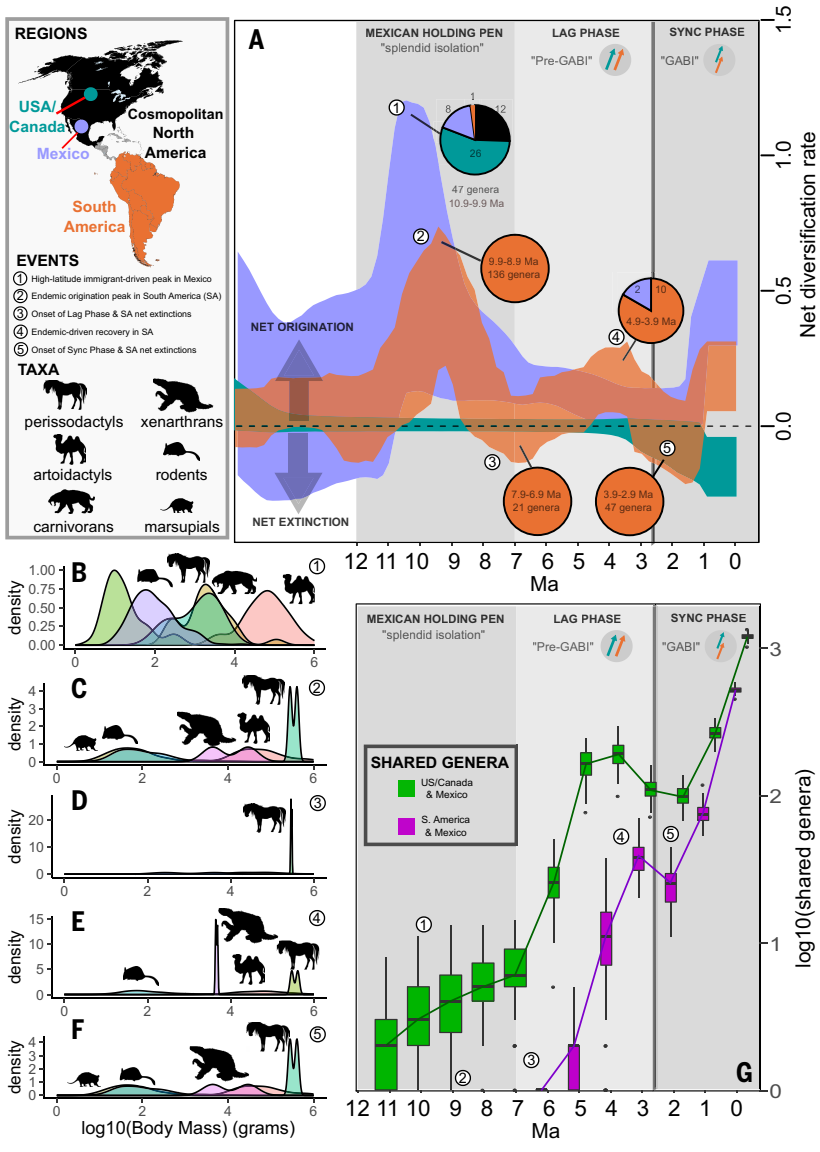

Fig. 1. A triphasic model of the GABI. (**A**) Geographic composition of net originations and extinctions during the past 12 million years, with five characteristic events of the holding pen, lag phase, and sync phase periods highlighted. (**B**) Body mass distribution of event 1 clades. (**C**) body mass distribution of event 2 clades. (**D**) Body mass distribution of event 3 clades. (**E**) Body mass distribution of event 4 clades. (**F**) Body mass distribution of event 5 clades. (**G**) Shared genera between the US or Canada and México versus between México and South America over the past 12 million years. The initiation of conventional GABI at ~2.8 mya is marked by a vertical dark gray line. [Organism silhouettes were downloaded from https://www.phylopic.org/; for attributions, see table S5.]

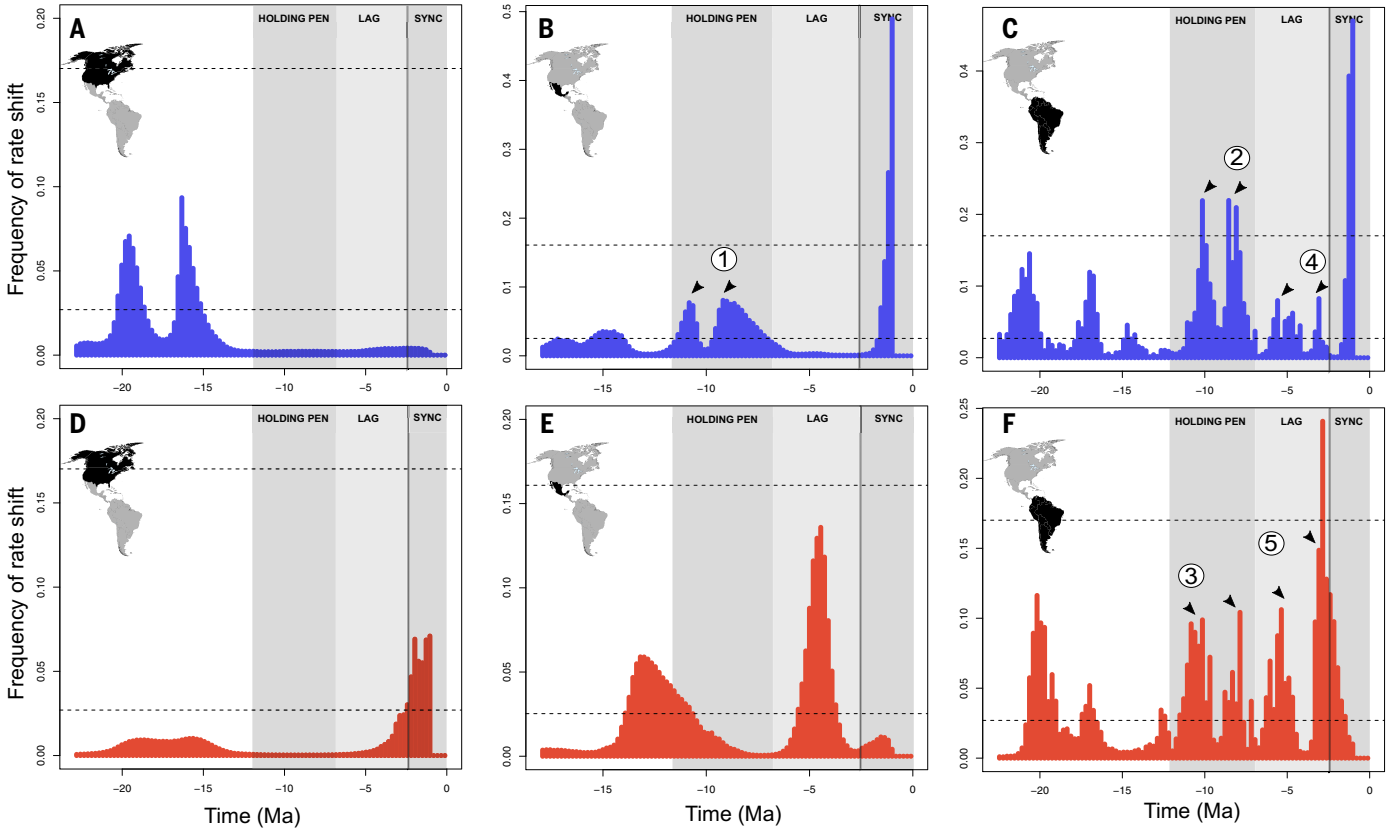

Fig. 2. Frequencies of rate shift in originations and extinctions estimated using a time-variable Poisson process model. (**A**) US and Canada originations. (**B**) México originations. (**C**) South America originations. (**D**) US and Canada extinctions. (**E**) México extinctions. (**F**) South American extinctions. Model input includes 100 resampled replicates (see the materials and methods) based on all occurrence data pulled from the Paleobiology Database (https://paleobiodb.org/#/) and those developed in this study. Arrow pairs indicate consecutive excursions of rate shifts marking net origination or extinction peaks. Numbering refers to the five major events explained in Fig. 1. The initiation of conventional GABI at ~2.8 mya is marked by a vertical dark gray line.

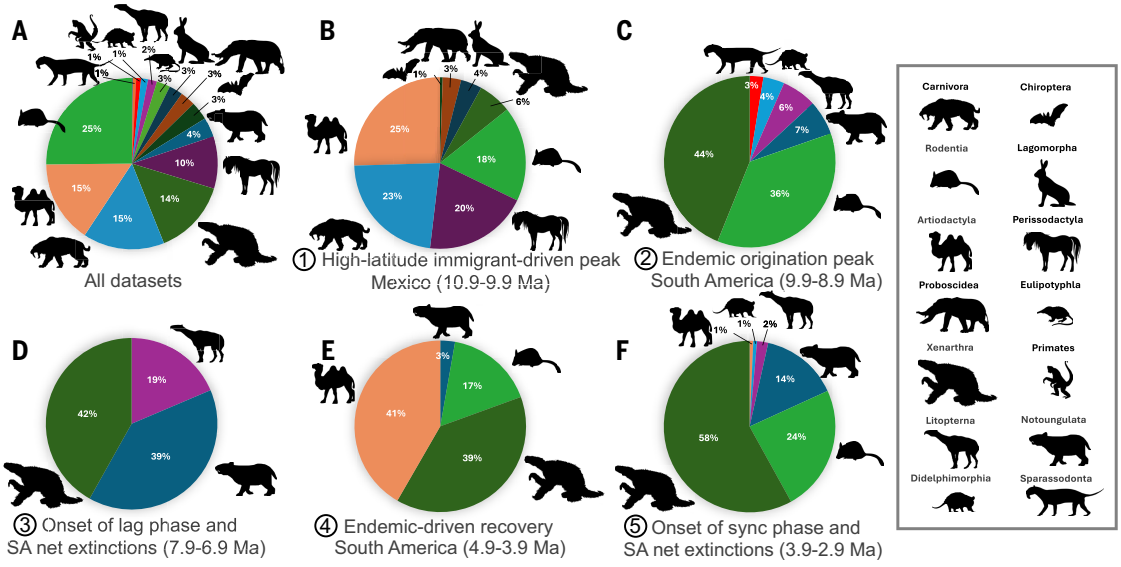

Fig. 3. Pie charts of taxonomic representation for all taxa sampled in each indicated geographic region and time interval during five diversification events leading up to interchange pulses. (**A**) Order-level proportions in the total fossil dataset. (**B**) High-latitude immigrant-driven origination peak in México during the holding pen phase. (**C**) Endemic origination peak in South America during the holding pen phase. (**D**) Net extinction event in South America at the onset of the lagging dispersal phase. (**E**) Endemic-driven recovery in South America during the transition from the lag phase to the sync phase. (**F**) Net extinction event in South America at the onset of the continentally synchronized dispersal phase. For definitions of the phases, see Fig. 1. [Organism silhouettes were downloaded from https://www.phylopic.org/; for attributions, see table S5.]

in the late Miocene holding pen relative to their representation during later dispersals (Fig. 3), but we observed a significant body size increase in sloth species from south to north, indicating that large body size, or the propensity to evolve large body sizes, facilitated cross-continental dispersals (figs. S11 and S14). In this regard, sloths were preadapted to succeed as one of the earliest cross-continental dispersers, given the clade's increased body size and size range evolution initiated in the late Miocene (*30*). Coupled with large body size, there is also a tendency for more dry environment–adapted taxa to appear in the holding pen compared with other major known pre-GABI events (Fig. 1, B to F; figs. S12 and S13; and tables S3 and S4). Expansion of savannah and grassland habitats and peaking of mammal richness in temperate North America may have evolutionarily enhanced the dispersal capability of those taxa as their habitats expanded southward (*29*). These data support the view that ecological barriers between low-latitude North America and South America served as dispersal filters (*4*) on top of the occasional availability of other dispersal mechanisms during the pre-GABI biogeographic expansions.

Geographic range expansion and latitudinal time lag

Between 7 and ~2.8 mya, temperate and low-latitude North America experienced an acceleration in connectivity coincident with the rapid expansion of savanna ecosystems (*31*). Locally, the increased importance of C_4 plants in holding pen ecosystems by 7 mya is evidenced by paleosol carbon isotope ($\delta^{13}C$) data (supplementary text and fig. S15) during a time when net diversification rates remained low (Fig. 1A). This suggests elevated homogenization of mammal genera in North America during this time period, potentially related to the establishment of modern-day landscape topology and the increased availability of north-south savannah corridors in the coastal, basin and range, and great plains provinces across North America (*32*–*34*). México and South America also became connected during this time but lagged behind the North American patterns both in pace and magnitude (Fig. 1). During the same phase, net extinction of endemic mammal clades in South America was evenly distributed relative to available source taxa (Figs. 1 and 3D and see "event 3" in fig. S10). This supports Vermeij's (*3*) hypotheses, which suggest that the GABI were asymmetrical, likely due both to the greater adaptive capabilities of successfully dispersing species and to the fact that endemic biotas experienced high extinction rates before the onset of the interchange (particularly in South American native ungulates; Fig. 3D). Low but positive net diversification rates followed, indicating that taxon replacement by in situ cladogenesis was supplemented by the addition of new taxa in the ecosystem from northern immigrant clades. The continuous presence of a north-to-south latitudinal lag in range expansion until ~3 mya supports the persistence of the holding pen partially through the Pliocene (5.3 to 2.6 mya).

Despite multiple documented arrivals of interchange immigrants before the Isthmus of Panama land-bridge formation sensu stricto, the presence of a Miocene (before 5.3 mya) overland corridor through the Panama Arc remains controversial (*8*, *15*, *16*, *35*, *36*). In addition to geological data cited in favor of (i) the middle Miocene "old Isthmus" hypothesis (*16*, *37*, *38*), other mechanisms previously proposed to explain pre-GABI dispersals include (ii) long-distance dispersal by rafting over oceanic waters (*8*, *11*, *39*) and (iii) alternative routes through the Caribbean Islands [the GAARlandia hypothesis (*17*)]. Our findings are compatible with the presence of both rafting and alternative routes of pre-GABI dispersals. The onset of the lag phase recovered in our analyses (Fig. 1 and fig. S8) further coincided with the time interval of the first appearance of C_4 grasses in South America, suggesting that the selective dispersal of North American mammals may be associated with the expansion of grasslands on both continents (*40*).

Finally, the replacement of endemic South American taxa was not absolute; new occurrences in South America during a net diversification event 5 to 4 mya included genera in endemic clades such as notoungulates, sloths, and glyptodonts, suggesting that the ecosystem was partially stabilizing (Fig. 3E) before the start of the conventional interchange interval (*41*). These distinct differences between pre-GABI and GABI phases are incompatible with the presence of persistent pre-Pliocene overland corridors implied by the old Isthmus hypothesis.

An expanded triphasic model

The presence of an old Mexican holding pen supports the conclusion that no viable land bridge was present for most mammal clades, both North and South American, before 7 mya. However, our findings do not contradict molecular divergence–based migration rate through time estimates presented by Bacon *et al*. (*15*), who identified accelerated rate values for temperate North and South American mammals starting around 7 to 6 mya. The observed latitudinal lag in connections between 7 and 3 mya, but not from ~2.8 mya on, further suggests that a dispersal mechanism distinct from the main interchange pulses is necessary to explain the presence of pre-GABI dispersals. Dispersing and extinct taxa during the lagging phase are evenly sampled from a cladistic perspective (Fig. 3 and fig. S10) and favor intermittent land corridor availability and/or climatic control rather than waif dispersal as a major dispersal mechanism, following the expectation of higher taxonomic biases in chance dispersal mechanisms relative to source faunal composition (*42*). Marine geochemistry patterns suggest distinct and complex deep- and shallow-water phases of Panamanian archipelago formation 7 to 2.8 mya, preceding complete Isthmus of Panama formation (*9*). This conclusion supports the view that a lagging dispersal phase reflected the occasional presence of an as-yet uncharacterized combination of tectonic, volcanic, and/or climatic mechanisms leading to one or more temporal windows of dispersal opportunity (*15*).

The recorded holding pen taxa were not a random subset of more broadly available source taxa in North America from a life history perspective; rather, they tend to be from larger-bodied clades and/or from more drought-adapted species than the broader global mammal biodiversity (Fig. 1, B to F; figs. S12 and S13; and tables S2 to S4). If living taxa serve as an informative model of past ecological adaptive events (*43*), then this would suggest that there may be selective advantages to having evolutionary flexibility in body size and/or drought tolerance during viable dispersal windows regardless of the mode of intercontinental dispersal. A potential lack of such evolutionary flexibility in some South American taxa may in part explain their disadvantage at dispersing northward and also surviving competition vis-a-vis immigrant taxa to the southern continent. Previously documented selective extinction of South American taxa during GABI on that continent, rather than higher dispersal or diversification rates of immigrants from the north, is consistent with this interpretation of asymmetric evolutionary viability (*3*, *44*). North America's deep history of sustained and asymmetric influx of immigrants from Eurasia during the late Cenozoic through Beringia may have produced dispersal-selected taxa such as the Eurasian immigrant-derived predators *Amphimachairodus* and *Huracan* (*Agriotherium*) in México (*45*–*47*). This history of faunal competition and selection was unfamiliar to their isolated South American counterparts and could have primed some North American taxa for dispersal success (*2*, *15*, *48*–*50*). Nevertheless, major questions remain as to how the macroevolutionary and ecological characteristics of dispersing taxa interacted with shifting tectonic configurations in the development of the Panama arc in early pre-GABI dispersals (*8*, *15*).

By the late Miocene, tooth enamel $\delta^{13}C$ data from Mexican herbivorous mammals suggest adaptation to C_3, mixed, and C_4 plant food sources, preceding the major interchange pulses starting at ~2.8 mya and consistent with our prolonged holding pen model (*51*). Herbivore niche partitioning among C_3, mixed, and C_4 diets in South America (Argentina) supports similar adaptations in some southern mammals (*52*). Paleosol carbonate $\delta^{13}C$ data suggest that C_4 grasses were present, at least locally, in Central México ~10 to 7 mya (fig. S15). A key unresolved question raised by these data is the role that vegetational and environmental transition zones played in defining the southern boundary

of the holding pen (*4*) and if the pen was a predecessor of the modern Nearctic-Neotropical transition zone proposed by Wallace (*53*), located between the Tropic of Cancer and the Trans-Mexican Volcanic Belt (*54*, *55*). Evidence for increasing provincialism of mammal faunas in central America over the course of the Neogene is suggestive but inconclusive (*56*). More geochronologically well-constrained central American paleontological data are required to identify and characterize the critical transition zone between southern México and northern South America during both the GABI and pre-GABI phases.

In summary, these findings support a broader temporal framing of the GABI and encourage a reevaluation of the role of latitudinal gradients in the timing and magnitude of terrestrial biotic events preceding and during the interchange. Analyses of the fossil record highlight distinctive biogeographic events across multiple pre-GABI phases and support the inference that low-latitude North America has been a biogeographic pacemaker since 10 mya. These conclusions underscore a prolonged prelude to the biotic interchange centered around a substantially older Mexican holding pen than previously recognized and challenge the hypothesis that the Isthmus of Panama formed by 10 mya.

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ACKNOWLEDGMENTS

We are grateful to J. Liu for discussion about GABI freshwater fishes, the overall framework of this research, and comments on previous drafts; S. Finnegan for discussion about marine assemblages in the Caribbean and eastern Pacific; C. Marshall for general discussion and wordsmithing help; the contributors and curators of the Paleobiology Database (https://paleobiodb.org/#/) for providing the community resources that made the analyses possible; and the artists for providing mammal silhouettes through https://www.phylopic.org. Stable isotope analysis was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement DMR-1644779 and the state of Florida. Data described in the paper are archived as accompanying data files. New fossil specimens used in this study are housed in and accessible through the Museo de Paleontología, Instituto de Geociencias (UNAM), in Juriquilla, Querétaro, México; for specimen catalog numbers, see data S2. **Funding:** This work was supported by the National Science Foundation (grant EAR 2102772 to Z.J.T., grant EAR 1949814 to Y.W., and grant EAR 1949742 to X.W.). **Author contributions:** Z.J.T.: conceptualization, design, funding acquisition, data collection, data analysis, manuscript writing, manuscript editing; L.X.K.: data collection, data analysis, manuscript editing; A.P.-C.: data collection, data analysis, manuscript editing; O.C.-C.: conceptualization, design, funding acquisition, data collection, manuscript editing; J.J.A.-G.: data collection, manuscript editing; Y.W.: conceptualization, design, funding acquisition, data collection, data analysis, manuscript editing; J.C.C.-A.: data collection, manuscript editing; C.H.: data collection, data analysis, manuscript editing; H.G.M.: data collection, data analysis, manuscript editing; X.W.: conceptualization, design, funding acquisition, data collection, manuscript editing. **Competing interests:** The authors declare no competing interests. **Data, code, and materials availability:** All data are available in the main text or the supplementary materials.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.aef2893
Materials and Methods; Supplementary Text; Figs. S1 to S15; Tables S1 to S5; References (*57*–*144*); MDAR Reproducibility Checklist; Data S1 to S8

Submitted 19 January 2026; accepted 28 April 2026

10.1126/science.aef2893

A 10-million-year biogeographic holding pen primed the Great American Biotic Interchange

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Science **393** (6810), . DOI: 10.1126/science.aef2893

Editor's summary

For millions of years after the breakup of the supercontinent Gondwana, South America was disconnected from other landforms, a time period that has been referred to as its "splendid isolation." This isolation allowed for the evolution of mammals with distinctive features, including xenarthrans, sprassodont carnivores, and notoungulates. The formation of the Panamanian land bridge facilitated connection of North and South American faunas, with a bias from north to south. Tseng *et al*. looked at fossils from low latitudes in México and found that northern mammals accumulated in the region before the connection (see the Perspective by McGuire and Williams). This allowed for more rapid dispersal south and further supports that the land bridge first formed during the Pliocene rather than the Miocene. —Sacha Vignieri