

## REVIEW

## ECOLOGY

## Formation of the Isthmus of Panama

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The formation of the Isthmus of Panama stands as one of the greatest natural events of the Cenozoic, driving profound biotic transformations on land and in the oceans. Some recent studies suggest that the Isthmus formed many millions of years earlier than the widely recognized age of approximately 3 million years ago (Ma), a result that if true would revolutionize our understanding of environmental, ecological, and evolutionary change across the Americas. To bring clarity to the question of when the Isthmus of Panama formed, we provide an exhaustive review and reanalysis of geological, paleontological, and molecular records. These independent lines of evidence converge upon a cohesive narrative of gradually emerging land and constricting seaways, with formation of the Isthmus of Panama *sensu stricto* around 2.8 Ma. The evidence used to support an older isthmus is inconclusive, and we caution against the uncritical acceptance of an isthmus before the Pliocene.

## INTRODUCTION

One hundred and fifty years ago, the striking similarity of marine animals on either side of the Isthmus of Panama was sufficient to convince naturalists that a seaway had once flowed between the Pacific Ocean and the Caribbean Sea (fig. S1) (1, 2). Formation of the Isthmus was a pivotal event, driving global oceanic reorganization and major biotic change on land and at sea. Populations of marine organisms divided by the rising land forged separate evolutionary paths in response to new and contrasting environments, and the timing of their divergence is now used to calibrate rates of molecular evolution (3, 4). The land bridge joined North and South America, permitting interchange of previously isolated terrestrial organisms with varying levels of success (5), deeply influencing today’s continental flora and fauna (6–8).

In the 1970s, high-resolution paleoceanographic data available from deep-sea cores began to show that an isthmus, defined by the *Oxford English Dictionary* as a “narrow portion of land, enclosed on each side by water, and connecting two larger bodies of land,” was in place only relatively recently, around 3 million years ago (Ma) (9, 10). This date has been accepted for over 40 years (11–13) but has recently been contested by interpretations that the Isthmus formed millions of years earlier. Bacon *et al.* (14) proposed that there may have been an initial land bridge as early as 23 Ma and later that the “Isthmus was formed” between 10 and 6 Ma, whereas Montes *et al.* (15) concluded that the Central American Seaway had disappeared by 15 to 13 Ma, stating that the Isthmus of Panama had formed at that time. If true, these new interpretations would revolutionize our understanding of the timing and causal relationships among environmental, ecological, and evolutionary change in the region. Some researchers have already accepted the new dates and called for major revisions of our understanding of global paleoceanographic and climate change, an alternative explanation for the Great American Biotic Interchange, and a compilation of new rates of molecular evolution (16–22).

However, there remains considerable conflicting evidence as well as confusion within the geological and biological communities about the proposal for an “old” isthmus. To bring clarity to the topic, we combine an exhaustive review with new analyses of geological, oceanographic, molecular, and paleontological records.

## GEOLOGICAL RECORDS

Formation of the Isthmus of Panama involved subduction of the Pacific-Farallon Plate beneath the Caribbean and South American plates, ultimately driving the development of a volcanic arc on the trailing edge of the Caribbean Plate. This initial Panama Arc began to form approximately 73 Ma (23) as the Caribbean Plate moved eastward, arriving at its current position by ~50 Ma. The North and South American plates continued to move westward past the Caribbean Plate after this time. In addition to their east-west (strike-slip) motion, the South American and Caribbean plates also acquired a north-south component of convergence, leading to the collision of the Panama Arc with South America. This collision drove uplift in both the Northern Andes and the Panama Block, forming the North Panama Deformed Belt and ultimately the Isthmus of Panama (24).

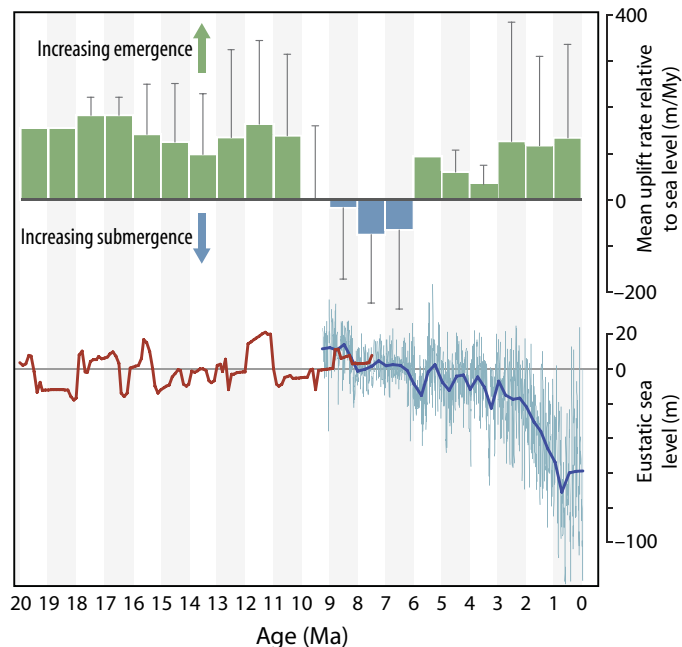
The Panama Arc is mostly composed of subduction-derived granitoids and associated volcanic rocks. Some early arc basement massifs appear to have been emergent since the Eocene as characterized by cooling below 200°C at that time, with continued exhumation and cooling episodes at 25 to 20 Ma, and at 12 to 6 Ma to less than 40°C (24–26). Between 25 and 23 Ma, the type of volcanic arc activity underwent a distinct change from hydrous mantle wedge-derived magmatism to localized extensional magmatism, indicating that the arc had impinged on South America (24, 27). Subsequently, the Panama Arc

underwent uplift at relatively moderate but constant rates (Fig. 1). As expected, uplift rates were generally higher in the Darien Basin (table S1), closest to the initial collision with the South American Plate. By 15 Ma, continued collision led to a transition from generally deepwater biogenic to siliciclastic sedimentation (28). Between 9 and 6 Ma, rates of paleobathymetric change in sedimentary sequences reveal significant deepening across the Panama Arc (Fig. 1) (28). Eustatic sea-level rise could in part be the cause of the deepening during the 9 to 8 Ma interval (Fig. 1), but later deepening cannot be explained by sea-level change and must therefore have been due to subsidence. The deepening event is most pronounced in the Bocas del Toro and Canal basins of Panama (table S1), suggesting that the cause may have been related to local tectonic extension (24), which would explain why adjacent crystalline massifs continued to be exhumed at the same time (25, 26). At around 6 Ma, the Panama Arc began rising again (Fig. 1) and has continued to do so until the present day. Arc uplift combined with sea-level falls driven by the expansion of the Greenland ice sheet around 3 Ma (29) and the establishment of repeated Pleistocene glaciations beginning at 2.6 Ma (Fig. 1) resulted in the land bridge connecting North and South America.

Montes *et al.* (15) argue that Eocene zircons found in middle Miocene river-borne sediments of Colombia's lower Magdalena Basin have a U-Pb age fingerprint that is uniquely "Panamanian" and must therefore have arrived, by river, from the Panama Arc. Thus, they conclude that the Central American Seaway must have "disappeared" by the middle Miocene. However, several other lithologic units could have been the source of the zircons found by Montes *et al.* (15) in the lower Magdalena Basin (Fig. 2). More than 30 Eocene localities, some containing zircons with U-Pb ages in the exact same age range

as those used by Montes *et al.* (15), have been identified in the Norandean region of South America (table S2). The two most likely alternative source regions are (i) a Paleocene-Eocene volcanic arc in the Central Cordillera (30) and (ii) the Sierra Nevada de Santa Marta (table S2 and Fig. 2). The true extent of Eocene zircons in the region therefore categorically negates the assertions of Montes *et al.* (15) that "there are no igneous bodies of that age in the northern Andes" or that the "Panama Arc and old Andean terrains are mutually exclusive geochronological domains." It is therefore unnecessary to invoke a land connection to explain the existence of Eocene zircons in Miocene sediments in the lower Magdalena Basin.

In addition, the assumption by Montes *et al.* (15) that a well-established Miocene fluvial connection existed between Panama and South America disregards the sedimentary record in the Urabá-Atrato-San Juan Basin that topographically separates Panama and South America (Fig. 2). These basins contain ~2500 m of marine sediments, transitioning from deep water in the Eocene and Oligocene to shallow water in the Miocene and Pliocene, and which were overlain after 3.7 to 3.1 Ma by terrestrial sediments (31–34). Detailed surveys of the region using surface mapping, radar-derived topographic data, exploratory well logs, and seismic cross sections, as well as gravimetric and magnetic surveys, clearly show that these sediments in the Atrato Basin extend into the Urabá Basin, entirely unaffected by the Cuchillo Hills (34). The Cuchillo Hills were therefore always islands in the Atrato sea, and marine connections penetrated the Dabeiba Arc forming a marine seaway until the Pliocene (Fig. 2 and fig. S2), refuting the assumption of a complete barrier by Montes *et al.* (15). A 150-m rise in relative sea level would be sufficient to flood the Urabá-Atrato-San Juan Basin and

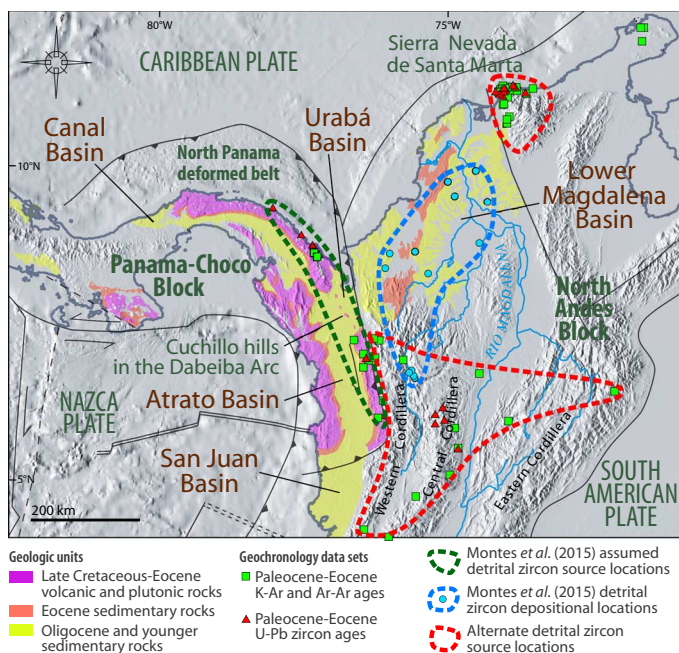


**Fig. 1. Uplift of the Isthmus of Panama and global sea levels over the last 20 My.** Rates of uplift are estimated from changes in the age and depth of deposition of sedimentary units across the Panama Arc (table S1) and are therefore relative to sea level. Eustatic sea-level estimates (light blue and dark red lines) from the study of Miller *et al.* (131). The dark blue line indicates values averaged within time bins of 250 thousand years (ky) for the 0 to 9 Ma record.

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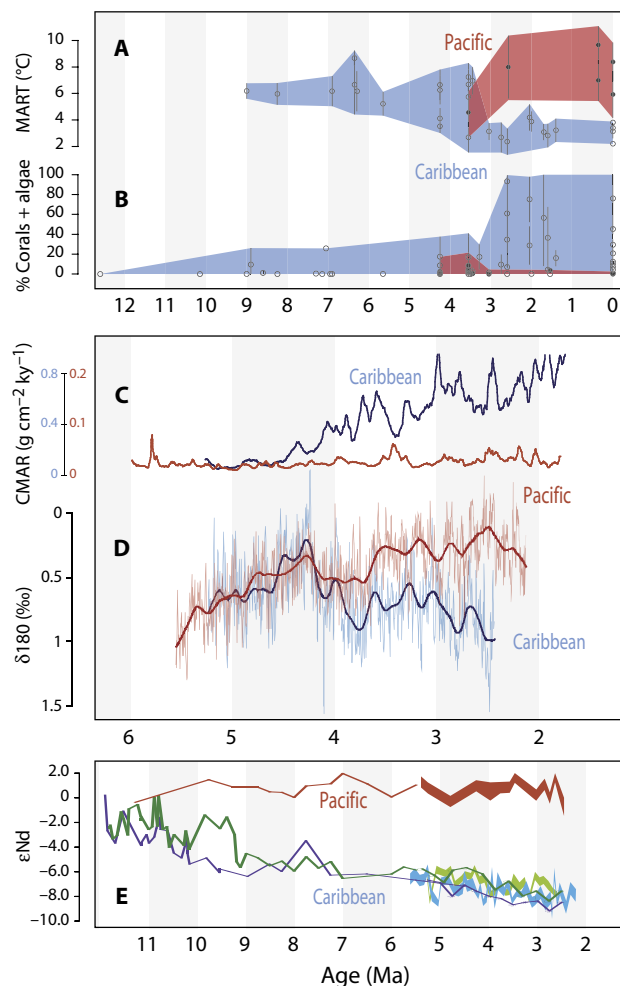
**Fig. 2. The Isthmus of Panama and northwestern Colombia with locations of some crystalline rocks (igneous and metamorphic) with reported Paleocene-Eocene radiometric ages (squares and triangles).** Montes *et al.* (15) proposed that Eocene detrital zircons in middle Miocene sediments of the Magdalena Basin (blue dots) could only be from plutonic rocks of the Panama Arc (dark green outline). However, several other rock units (for example, Western, Central, and Eastern Cordilleras plus the Sierra Nevada de Santa Marta; red outlines and table S2) exhibit radiometric ages in the same interval and therefore must also be considered as potential sources. From previous studies (24–26, 30, 31, 132).

the Canal Basin (fig. S2). Such a rise is easily accounted for by arc uplift and eustatic sea-level changes over the last 3 million years (My) (Fig. 1).

### MARINE PALEONTOLOGICAL RECORDS

Formation of the Isthmus of Panama is recorded by changes in the chemistry, composition, and structure of sediments and fossils in deep sea and coastal rock records across the Caribbean and eastern Pacific. In the deep ocean, divergences in neodymium isotopes (Fig. 3E) (35) and benthic foraminifera  $\delta^{13}\text{C}$  (36, 37) between Caribbean and Pacific sites, as well as an abrupt increase in the Pacific carbonate compensation depth, provide strong evidence that deepwater connection was shut off between 12 and 9.2 Ma. At this time, the deepest part of the sill of the Panama Arc must have shoaled to less than 1800 m and was perhaps as shallow as 1200 m (38).

Ocean circulation models suggest that constriction of interoceanic straits should have caused the Atlantic to become saltier because the trade winds transported moisture into the Pacific (39). In turn, the increase in North Atlantic salinity should have driven increased thermohaline overturning in the North Atlantic and introduced low- $\text{CO}_2$  and low-nutrient waters into the deep Atlantic by overturning nutrient-poor surface waters (39–42). As predicted by these models, western Atlantic surface water salinity began to diverge from eastern Pacific values about 4.6 Ma and reached modern Caribbean values by about



**Fig. 3. Timing of divergence between Caribbean (blue) and Tropical Eastern Pacific (red) environments and ecologies in coastal, shallow, and deep waters.** (A and B) Estimated mean annual range of temperature [MART; a proxy for strength of upwelling estimated by measuring zooid size variation in fossil bryozoan colonies (133)] (A) and the relative skeletal weight of corals and coralline algae (68, 134) (B) in replicated bulk samples from coastal shelf sediments on the Isthmus of Panama. (C and D) Rates of accumulation of carbonate (CMAR) in deep-sea sediments (C) and estimated surface water salinity (reflected in the oxygen isotope record) (D) in Caribbean and Pacific surface waters. (E) Neodymium (Nd) values from fish teeth and foraminifers in Pacific and Caribbean basins (35, 38).

4.2 Ma (Fig. 3D) (36, 42). Deep Caribbean  $\delta^{13}\text{C}$  increased toward modern values at the same time, in response to flooding of the Caribbean by nutrient-poor North Atlantic Deep Water (36, 37, 43). Finally, Caribbean carbonate preservation improved at 4.6 Ma, likely reflecting the replacement of corrosive Antarctic Intermediate Water by North Atlantic Deep Water as overturning increased in the northern North Atlantic (Fig. 3C) (43). The development of a large salinity contrast between the Caribbean and eastern Pacific (Fig. 3D) is a particularly strong indication that the Panama Arc was mostly emergent by 4.2 Ma (40, 44).

Nonetheless, vigorous exchange of near-surface waters between the oceans continued as demonstrated by similarities in the radiolarian (45), foraminiferal (9, 10, 46), and nannoplankton coccolithophore (47) communities of the Caribbean and Tropical eastern Pacific. Radiolarian



assemblages in the Gulf of Panama and the presence of the foraminifer *Neogloboquadrina pachyderma* (sinistral coiling) in the Western Caribbean are distinct indicators of increased upwelling starting about 4.2 Ma that have been interpreted as shoaling of the Caribbean-Pacific sill (45, 48). The temperature gradient in the eastern Pacific thermocline also steepened between 4.2 and 3.8 Ma, consistent with increased coastal upwelling (41, 49). The Indo-Pacific planktonic foraminifer, *Pulleniatina*, and a suite of menardellids and other foraminifer species disappeared from the Caribbean between 3.5 and 3.0 Ma in a pattern indicative of the shoaling of the Panama Arc (9, 10, 46). Coccolithophore communities were similar between eastern Pacific sites and the southern Caribbean up until between 3.65 and 2.76 Ma and then diverged as the Panama Arc cut off surface water flow between the oceans (47). The redevelopment of similar salinity and sea surface temperatures between the Tropical Eastern Pacific and Caribbean suggests temporary breaching of the Isthmus as late as 2.45 Ma (44).

In fossil sediments in the area around the Panama Canal, coral reefs, mangroves, and deltaic sediments (50–52) demonstrate that parts of the Panama Arc were emergent since at least 30 Ma. Until around 4 Ma, there was little taxonomic or ecological difference in shelf benthic and nektonic communities between the Tropical Eastern Pacific and the Caribbean (53–56), demonstrating easy movement of water carrying larvae or adults between the oceans (57). Around 4 Ma, Caribbean-wide decline in nutrients drove a profound change in the structure of Caribbean coastal communities and environments (Fig. 3, A and B) (58–62) and the life histories of the animals that inhabited them (63–65). Oligotrophic conditions allowed reef-building corals and their associates to proliferate (Fig. 3B) (59, 66, 67). A regional extinction across the Caribbean between 4 and 2 Ma (54) was highly selective against animals suited to high planktonic productivity (60, 61, 63, 68), implicating declining nutrients due to the restriction of Pacific waters entering the Caribbean, most likely caused by the emergence of the Panama Arc and formation of the Isthmus.

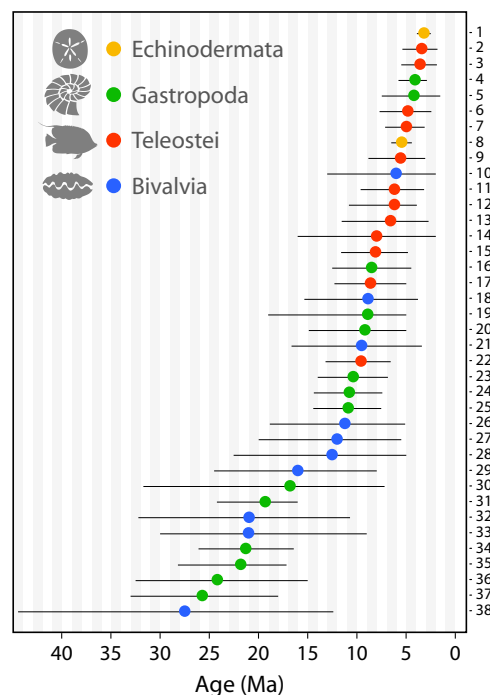
### MARINE MOLECULAR RECORDS

Barring some exceptional cases of migration around the southern tip of Africa (69) and transits of organisms through the Panama Canal (70), molecular divergence between eastern Pacific and western Atlantic shallow-water marine organisms provides evidence of when the last interoceanic connections were severed. To estimate dates from molecular divergence, it is necessary to (i) determine the phylogeny of a group, to identify sister species in either ocean, and (ii) estimate the rate at which their molecules have evolved. Most calibrated phylogenies of marine organisms have assumed that the Isthmus closed at 4 to 3 Ma (3), but it would be circular to use these estimated rates of molecular evolution to date Isthmus formation. In an attempt to remove this circularity, Bacon *et al.* (14) assumed a “universal” rate of mitochondrial DNA (mtDNA) divergence of 2% per million years. However, this is inappropriate, because rates of molecular evolution vary substantially between clades (71–74); two approaches are taken here.

In our first approach, we estimated times of separation between Atlantic and Pacific sister species from molecular phylogenies that have been calibrated by fossils at one or more nodes and are therefore independent of emergence of the Isthmus. As we were interested in the time of the most recent common ancestor of species, rather than of particular genes (which coalesce farther back in time than popula-

tions), we further restricted our choice of phylogenies to those based on a combination of mtDNA and nuclear DNA regions (see text S1 for details). Of the 38 comparisons based on fossil-calibrated phylogenies, 26 (68%) produced estimates of separation that occurred more recently than 12 Ma (Fig. 4), demonstrating that seawater passages of sufficient width and flow to convey larvae or adults between the Pacific and Caribbean continued after this date. Other comparisons indicate older splits, which may have occurred either because of gradual isthmus uplift or resulted from extinction in one ocean of one daughter species of a common ancestor originally spread in both oceans, creating the false impression that the extant remnants were actually sister species (3, 75). The four most recent divergences in our analysis are the cone snail *Conus* at ~4.1 Ma [95% highest posterior density (HPD), 5.80 to 2.90 Ma], the grouper *Mycteroperca* at 3.58 Ma (95% HPD, 5.51 to 1.90 Ma), the butterflyfish *Chaetodon* at ~3.4 Ma (95% HPD, 5.40 to 1.80 Ma), and the sand dollar *Mellita* at ~3.21 Ma (95% HPD, 3.91 to 2.51 Ma) (table S3). The interoceanic mixing necessary to produce these dates could not have occurred if the Isthmus of Panama had existed (76).

For our second approach, we tabulated divergence between members of transisthmian pairs that have been shown to be sister species by molecular phylogenies, but which lack fossil calibrations, to ask whether such divergence values are consistent with an old or a recent time of separation, given the rate of mtDNA evolution estimated from our fossil-calibrated phylogenies. Of the 95 divergences without fossil calibrations (table S4), 60 show mean Kimura two-parameter distances over all sampled genes of <10% (median divergence, 4.65%). If gene flow between these most recently separated sister species pairs



**Fig. 4. Bayesian estimates of median molecular divergence times of sister taxa on either side of the Isthmus of Panama.** Only species for which both nuclear DNA and mtDNA data were available and for which divergence ages were calibrated by the fossil record are included. Error bars represent 95% HPDs. Numbers refer to species pairs in table S3.

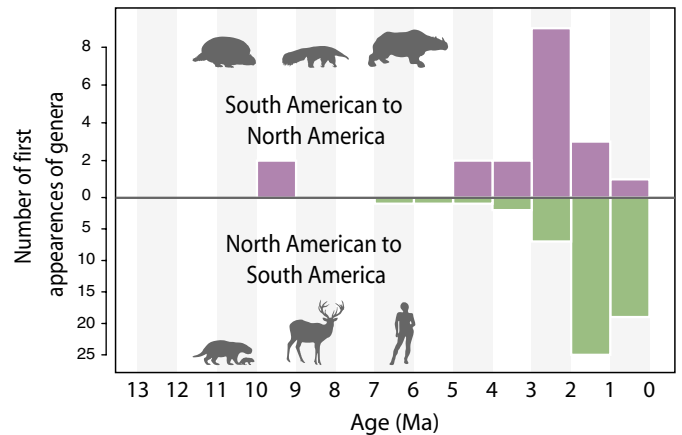
is assumed to have been interrupted 2.8 Ma, this value translates to a median divergence rate of 1.66% per million years over all genes and taxa. If, on the other hand, one assumes that the Panama Arc permanently blocked all genetic exchange from 23 to 13 Ma (15), the estimated median divergence rates would be 0.20 to 0.43% per million years. The median divergence rate of mtDNA evolution in transisthmian pairs with fossil-calibrated phylogenies is 1.06% per million years (table S4); thus, the rates that assume an old separation of the oceans are inconsistent with the dates derived from the fossils. It is very unlikely that evolution of mtDNA was accelerated exclusively in species that happen to have a fossil record.

Therefore, these molecular comparisons demonstrate interoceanic gene flow until approximately 3 Ma. In contrast, Bacon *et al.* (14) used a meta-analysis of molecular data to measure rate shifts in migration, observing concentrations of divergence “events separating marine organisms in the Atlantic and Pacific oceans at ca. 23 and 7 Ma,” then assuming that these provide evidence for early (but transient) formation of the Isthmus of Panama at these dates. Bacon *et al.*'s argument for the significance of the 23 Ma cluster is that there was a corresponding concentration of terrestrial divergences at ~20 Ma, implying a common geological cause. However, in addition to using an inappropriate universal rate of mtDNA divergence, a substantial number of published data sets were omitted (77), and data from marine organisms were limited to only COI with the argument that data from other genes are not as reliable (78), although most of their analyses of terrestrial organisms were based on other genes. When all of the missing marine molecular data were included, the proposed ~23 Ma marine event disappeared (Fig. 1) (77).

## INTERCONTINENTAL DISPERSALS OF TERRESTRIAL ANIMALS

The earliest appearances of migrating terrestrial animals in fossiliferous deposits demonstrate the timings of successful intercontinental dispersals and help illuminate when the Isthmus of Panama formed (Fig. 5). We limit our review only to fossils with confident taxonomic placement that come from well-dated geological units. Except for a South American salt water-tolerant crocodile (79) and a monkey (80), early and middle Miocene fossils uncovered in the Panama Canal region and the Gracias Formation in Honduras have so far been almost entirely North American in their affinities (51, 81, 82). Likewise, the middle Miocene La Venta Formation in Colombia (83), the late Miocene Urumaco Formation in Venezuela (84), and the Solimões Formation in Brazil (85) contain only South American taxa. Thus, the two continents apparently exchanged few nonflying vertebrates for over 40 My.

Around 9 to 8.5 Ma, the sloths *Thinobadistes* and *Pliometanastes*, derived from South American ancestors, appeared in North America (86, 87). Their success in the north, along with the arrival of the first South American edentate genera *Glossotherium* and *Plaina* in Mexico around 4.8 to 4.7 Ma (88, 89), is suggestive of their ecological, and therefore competitive, distinction from already established herbivorous mammals in North America (90, 91). The first record of North American immigrants in South America is the omnivorous endemic procyonid carnivore *Cyonasua* at ~7 Ma (92), followed by the endemic sigmodontine rodent *Auliscomys* at ~5 Ma (93), both of which are likely to represent much earlier migrations that have yet to be revealed



**Fig. 5. Frequency of appearances of immigrant vertebrate taxa or their oldest known descendants in opposing continents as observed in well-dated fossiliferous sediments in South and North America per million years.** See fig. S3 for further details.

in the fossil record. The South American flightless terror bird *Titanis* occurred in Texas between about 5.0 and 4.7 Ma (94). A close relative of the capybara, *Neochoerus*, is first recorded present in Mexico around 3.8 Ma, and *Glyptotherium* is also recorded in Mexico around the same time (fig. S3) (88, 89).

Some researchers have argued that dispersals of animals and plants between North and South America before 3 Ma indicate an early completion of a land bridge (14, 95). Bacon *et al.* (96), for example, estimated that *Copernicia* and *Pritchardia* palms dispersed between North and South America from 31 to 9 Ma, and thus concluded that the Isthmus of Panama had emerged in the Oligocene or Miocene. However, many kinds of plants and animals are known to have dispersed over ocean barriers with surprising frequency, often crossing gaps much wider than the widest plausible strait separating North and South America in the Miocene (97–99). Primates and rodents crossed the Atlantic in the Eocene (100). All the post-Eocene mammal lineages of Madagascar, including hippopotamuses, arrived from Africa (101, 102). Most of the (endemic) modern fauna of the West Indies is derived from overwater colonists (103). Salt water-intolerant amphibians crossed sea barriers numerous times (104, 105).

Rafting is the most likely method of dispersal of terrestrial organisms over water. Natural rafts of soil and vegetation that form when floods wash away parts of river banks are frequently observed far out at sea (106, 107), especially in areas receiving tropical storms, and these rafts might harbor enough food and fresh water to maintain animals for a journey of weeks or months. Rafts frequently reach very large sizes (video S1), and prevailing winds, currents, or storms can carry them relatively rapidly over long distances (101, 108). The Atlantic crossings by monkeys and rodents, which almost certainly occurred by rafting, indicate the great potential of this mechanism for long-distance dispersal.

Many of the mammals that traveled between North and South America before 3 Ma (for example, procyonids, sigmodontine, and caviomorph rodents; platyrrhine monkeys; and sloths) are members of groups that have reached islands by oceanic dispersal (101, 108–111). Hence, the early dispersal of these particular animals and not others actually argues in favor of separation of the continents. Considering how near the Panama Arc was to South America for 20 My, it is

surprising that there were so few successful dispersals of terrestrial animals, especially in the light of known oceanic dispersals over much greater distances around the world. Strong ocean currents may have created a formidable barrier similar to the Indonesian throughflow (112), which accounts for the Wallace and Lydekker lines (113, 114); this interpretation is supported by paleoceanographic models of large-scale interoceanic water exchange between the Pacific and Caribbean as the Isthmus of Panama shoaled (29, 40, 115). Alternatively, dispersals may have been frequent but mostly unsuccessful because of unsuitable ecological conditions and/or stiff competition from resident incumbent faunas (13, 116–118).

The Great American Biotic Interchange (119) is characterized by a surge in successful dispersals in both directions beginning around 2.6 Ma, traditionally defined as beginning with the arrival of the South American porcupine *Erethizon* in North America (116), and various members of the North American families Mustelidae, Canidae, and possibly Gomphotheriidae, along with the extinct horse *Hippidion*, successfully colonizing South America at the same time (fig. S3) (8). This wave of successful dispersals by many large mammals is widely considered convincing evidence that animals could, at this time, have walked dry-shod across a fully formed land bridge.

In stark contrast to the available fossil record of successful dispersals, Bacon *et al.* (14), using molecular divergences of terrestrial plants and animals, concluded that the Isthmus of Panama formed in fits and starts ~23 to 19 Ma and then again ~8 to 5 Ma. The former date coincides roughly with initial contact of the Panama Arc with South America (24), which would likely have increased the chance of successful overwater dispersals. However, the proposed peak in divergences ~8 to 5 Ma coincides with no known geological or environmental driver; on the contrary, interoceanic seaways were growing in size at this time (Fig. 1). The disagreement could potentially result from a dearth of tropical terrestrial fossil records (120) but more likely is caused by the inappropriate use of molecular divergences between terrestrial organisms as a tool to date a land bridge. As Daza *et al.* (121) noted, taxa begin to diverge when they are separated; they do not diverge when they spread from one place to the other, unless that event is immediately followed by isolation. Thus, unless isthmus formation were followed by reestablishment of seaways, the early divergences discussed by Bacon *et al.* (14, 96) are evidence for dispersal over water, which would provide a mechanism of isolation after colonization, rather than over land.

This effect also applies to dating based on the age of crown groups now endemic to separate continents, because their stems must have been isolated to diverge. Deep divergences between such biotas, if not caused by pre-isthmus dispersal over water, could reflect the formation of two clades within one of the continents, followed by crossing by one or both clades to the other continent at any time after a land bridge formed. Subsequent extinction of one clade in one of the two places would result in two sister clades, one in North America and one in South America, the separation of which would far pre-date the time of colonization. Thus, unlike fossils, which provide definitive evidence of the minimum time of arrival, the use of divergences between North and South American terrestrial clades is an unreliable tool for dating the formation of the Isthmus of Panama.

## SUMMARY

Establishing how and when the Isthmus of Panama formed is crucial for understanding the greatest “natural experiment” ever (122, 123).

Our reviews and new analyses show that, before isthmus formation, the Panama Arc existed as a semi-emergent island chain through which abundant seawater flowed from the Pacific into the Caribbean since at least 30 Ma. The arc collided, initially underwater, with South America around 24 Ma and has continued to do so to this day (24). Meanwhile, the largest of the interoceanic straits were more than 1200 m deep, permitting massive interoceanic seawater exchange until these deepwater passages were extinguished by 9.2 Ma (35, 38), after more than 15 My of collision and uplift. Proximity of emergent land on the Panama Arc to South America increased the probability that terrestrial animals and plants could disperse between the continents across the seaways. Land mammal dispersals began ~20 Ma and trickled on for a further 17 My, most likely via rafting. Shallow seawater continued to be exchanged between the oceans until ~4 Ma whereupon the number of diverging marine species peaked (Fig. 4), and the Caribbean underwent a profound environmental, ecological, and evolutionary transformation (Fig. 3) resulting from significant constriction of the interoceanic seaways. Despite the near completion of a land bridge, ample interoceanic gene flow continued until at least ~3.2 Ma, suggesting that strong currents passed through the straits into the Caribbean, as suggested by models of the shoaling Panama Arc (29, 40, 115). Strong interoceanic currents could also explain why surprisingly few terrestrial animals successfully dispersed across the straits in the Miocene and Pliocene (Fig. 5) [compare with the study of Wallace (113)]. Alternatively, host continents may have been competitively or climatically unsuitable to migrants (80, 116, 117).

Formation of the isthmus *sensu stricto* is pinpointed by (i) the absence of further gene flow between shallow marine animal populations after ~3.2 Ma (Fig. 4), (ii) the end of surface water exchange between oceans at 2.76 Ma based on marine plankton assemblages and surface ocean salinity contrasts (Fig. 3), and (iii) an observed acceleration in the rate of dispersal of terrestrial mammals between continents sometime just before 2.7 Ma (Fig. 5 and fig. S2). These events coincide with glaciations, first of Greenland, followed by the Eurasian Arctic, North-east Asia, and Alaska (124, 125), resulting in significant drops in sea level (Fig. 1). Uplift of the Panama Arc and falling sea level (Fig. 1) therefore worked in concert to form the Isthmus of Panama *sensu stricto* at this time (44). Interglacial sea-level rises may have breached the Isthmus temporarily (67), potentially permitting gene flow between the oceans. Ephemeral similarities in surface ocean salinity between the Caribbean and eastern Pacific suggest that short-lived breaching may have occurred as late as 2.45 Ma (44).

Our reviews reveal that evidence used to support an older isthmus is inconclusive. For example, zircons claimed to be uniquely Panamanian (15) could have arrived from several alternate (and less distant) sources in northwest South America. Equally, molecular divergences of terrestrial animals used to date the Isthmus (14) are a poor proxy for a land bridge, and marine molecular divergences that use universal rates (14) can be flawed. The campaign for a pre-Pliocene isthmus puzzlingly admits that seawater channels may have persisted into the Pliocene (14, 15, 78, 126), illustrating how the definition of an isthmus is far from a pedantic issue (12). In the Indonesian–Malay archipelago, for example, shallow and narrow seaways permit considerable oceanic throughflow (127) that maintains chemical balance between the Pacific and Indian oceans, controls monsoonal climate of the entire Indian Ocean region (112, 128–130), maintains liberal gene flow among a great many Indo-Pacific species, and limits intercontinental migration of terrestrial vertebrates between Asia and Australia (113). Given all

the available evidence, we strongly caution against the uncritical acceptance of the old Isthmus hypothesis (16–18, 22).

## SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/2/8/e1600883/DC1>

fig. S1. The Isthmus of Panama in Tropical America.

fig. S2. The current day Isthmus of Panama submerged under 150 m of relative sea-level rise.

fig. S3. Timing of successful terrestrial dispersals between the American continents as observed in the occurrence of fossil remains in the rock record of North and South America.

video S1. Large rafts form on the Río Chagres, Gamboa, Republic of Panama, on 8 December 2010.

table S1. Estimates of rates of uplift for the Panama Arc relative to sea level, using changes in estimated median depths and median ages of sedimentary units from previous studies (28, 135–149).

table S2. Compilation of Late Paleocene–Late Eocene ages for the Colombian Andes plotted in Fig. 2.

table S3. Median and 95% HPD intervals of the time at which members of clades spanning the Isthmus of Panama were separated from each other as estimated by BEAST [1] from phylogenies calibrated by fossils at one or more nodes.

table S4. Kimura two-parameter distance between mitochondrial genes of sister clades on either side of the Isthmus of Panama.

text S1. Estimation of date of splitting from molecular divergence.

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**Acknowledgments:** We thank A. Dornburg, A. Herre, C. McMillan, D. Piperno, D. V. Vliet, E. Tonni, E. Bermingham, E. Dillon, F. Rodriguez, F. Santini, H. Lotze, I. Rubino, J. Hodge, J. Measey, K. O'Dea, K. Effio, M. Miller, M. Pierotti, N. Parra, O. McMillan, S. Tierney, S. Williams, and W. Schwarzhans for valuable discussion, data, and assistance. **Funding:** This study was supported by the Smithsonian Tropical Research Institute to A.O., J.B.C.J., N.K., and H.A.L.; the NSF (EAR 1325683) to A.O., P.G.R.-D., and E.L.G.; the National System of Investigators to A.O.; the Secretaría Nacional de Ciencia, Tecnología e Innovación (Panamá) to A.O., H.A.L., and S.E.C.; the U.S. Geological Survey to R.F.S.; and the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) to A.L.C., G.M.G., E.S., and L.S. **Author contributions:** A.O., A.G.C., R.I.E., J.B.C.J., N.K., H.A.L., R.D.N., and S.A.R.-M. designed the research. A.O., O.A., M.-P.A., W.A.B., A.F.B., A.L.C., A.G.C., L.S.C., S.E.C., M.A.C., A.d.Q., H.D.-C., R.I.E., D.W.F., S.F., G.M.G., E.L.G., J.B.C.J., K.G.J., L.D.K., N.K., E.G.L., J.S.L.-P., H.A.L., P.B.M., R.D.N., N.D.P., P.G.R.-D., S.A.R.-M., E.S., L.S., R.F.S., J.A.T., G.J.V., and M.O.W. performed the research. A.O., L.S.C., S.E.C., R.I.E., H.A.L., P.B.M., and S.A.R.-M. contributed data. A.O., L.S.C., R.I.E., S.F., and H.A.L. analyzed data. A.O., D.W.F., P.G.R.-D., S.A.R.-M., and R.F.S. drafted figures. A.O., A.L.C., A.G.C., A.d.Q., R.I.E., D.W.F., H.A.L., R.D.N., S.A.R.-M., and M.O.W. wrote the paper. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 24 April 2016

Accepted 18 July 2016

Published 17 August 2016

10.1126/sciadv.1600883

**Citation:** A. O'Dea, H. A. Lessios, A. G. Coates, R. I. Eytan, S. A. Restrepo-Moreno, A. L. Cione, L. S. Collins, A. de Queiroz, D. W. Farris, R. D. Norris, R. F. Stallard, M. O. Woodburne, O. Aguilera, M.-P. Aubry, W. A. Berggren, A. F. Budd, M. A. Cozzuol, S. E. Coppard, H. Duque-Caro, S. Finnegan, G. M. Gasparini, E. L. Grossman, K. G. Johnson, L. D. Keigwin, N. Knowlton, E. G. Leigh, J. S. Leonard-Pingel, P. B. Marko, N. D. Pyenson, P. G. Ravello-Dolmen, E. Soibelzon, L. Soibelzon, J. A. Todd, G. J. Vermeij, J. B. C. Jackson, Formation of the Isthmus of Panama. *Sci. Adv.* **2**, e1600883 (2016).