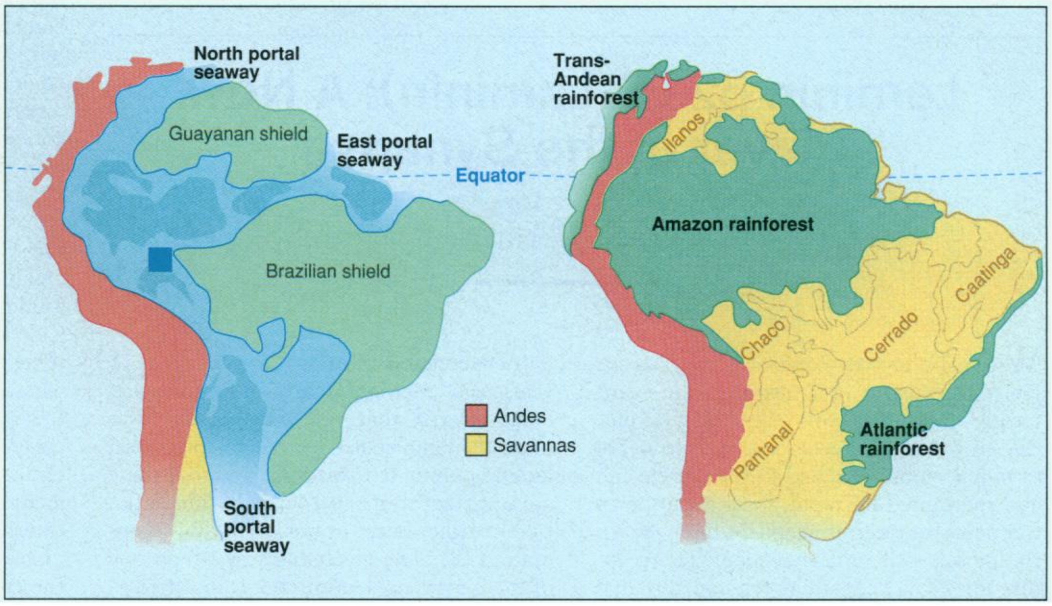
Biological Implications of the Middle Miocene Amazon Seaway

S. David Webb

Even today, although it is under siege, the Amazon Basin supports greater terrestrial biodiversity than any comparable area on Earth (*1*). For that reason its long-term history fascinates biologists and conservationists as much as it does geologists. Formerly, the essence of Amazon history was "stability." Rain forests were thought to have continuously clothed South America's equatorial lowlands since its Cretaceous separation from Africa about 80 million years ago, whereas equatorial Africa suffered bouts of aridity and other climatic deterioration that had reduced the diversity of its rain forest to roughly half that of Amazonia (*2*). In recent decades, however, geologists and biologists have documented a "dynamic" history for Amazonia. During nearly two million years of the Quaternary Period, as glacial climates became more zonal and more extreme, savannas expanded, forcing rain forests into refugia. Savanna versus rain forest dominance oscillated as semiarid glacial epochs alternated with humid interglacials such as the present (*3*). Thus, dynamism has supplanted stability as the evolutionary model to explain the rich neotropical fauna and flora. Until now, however, there have been very few data to say whether this dynamism extended back beyond the Quaternary.



Then and now. A comparison of proposed middle Miocene paleography and present terrestrial habitats of tropical South America.

In this issue of *Science*, a team of Finnish and Brazilian sedimentologists and paleontologists present evidence that the Amazon Basin was occupied by an interior seaway about 10 million years ago (*4*). Although the general concept of an Amazon Sea is not altogether new, the article by Räsänen *et al.* (see p. 386) is the first to marshal substantial evidence placing this interior sea in a testable time and place. In the southwestern frontier of Amazonian Brazil, one can see during the dry season the Solimoes formation, a tabular body of horizontal Miocene sediments, for several days while journeying along the Jurua, Purus, and other Amazon tributaries. Seven years ago, another group studied essentially the same sets of sediments and fossils but interpreted them as a vast interior lake, which they named "Lago Amazonas" (*5*). The two new clues to marine conditions are the tidal signature of sedimentary sets ("rhythmites") and the abundance of fossil sharks. More work is needed to confirm these preliminary data and to extend them geographically. Even though this sedimentary package seems extensive in the field, the studied area is small in comparison with that of the probable seaway. This endeavor is difficult not only because the study area is remote, but also because geological exposures are limited to riverbanks that are accessible only during the dry season, supplemented recently by road cuts along the trans-Amazonian highway.

It is revealing to compare the present Amazonian rain forest distribution with the probable Amazon Sea of the Miocene (see figure). Such interior seas are rare in today's world of low sea levels. The largest in North America is the Hudson Bay–St. Lawrence Seaway, but it is tiny compared to the Sundance Sea, which completely divided North America during the late Cretaceous. The figure indicates three possible portals of the probable Amazon Sea, to the north, to the south, and in the present course of the Amazon River to the east. Assuming that the topography was approximately the same as that of the present, these portals are credible, for today's headwater crossings from the Rio Negro northward into the Orinoco and from the Madeira southward into the Paraguay rise only to about 400 m.

The age of the probable Amazon Sea requires more precise dating. The mid-Miocene estimate for the western Amazon Basin depends on a short list of extinct land mammals collected mainly in deltaic facies that interfinger with the presumed marine sediments of the Solimoes Formation and Pebas beds. The small rodents and primitive nothrothere sloth could be Chasicoan (the next-older mammal age) rather than Huayquerian. This would date the high stand of the Amazon Sea at about 12 million years, thereby making it correlative with the age of Paranense Sea, well-documented in Paraguay and Argentina (*6*), and with the highest global sea levels (Serravallian stage) of the Miocene (*7*). The Huayquerian (about 10 million years ago), on the other hand, was a time of lower sea levels when two families of ground sloths colonized North America and extinct raccoon relatives reciprocally reached South America. The absence of marine mollusks and other invertebrates from Miocene sediments of the Amazon probably reflects subsequent dissolution by deep acidic weathering. This absence of marine invertebrate fauna is unfortunate because it could potentially provide an independent age estimate.

How does this new evidence of an extensive Amazon Sea in the middle Miocene

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relate to biological views of the Amazon biota? First, it explains why the Amazon River has an extraordinarily high diversity of endemic marine-derived species, much greater than that of the Congo or any other large river system. The introduction and isolation of such intriguing Amazon taxa as iniid dolphins, trichechid seacows, stingrays, and tilefish may now be attributed to the rise and fall of the Miocene seaway. Second, the rich terrestrial biota, for which the Amazon Basin is justly famous, is now seen to be drawn from three separate land areas (see figure). Each parcel of equatorial lowland provided an independent theater of evolutionary experimentation. Such division and coalescence conforms well to the model regarded by many evolutionists as the most productive "species pump." This new view of the rise and fall of the Amazon Sea extends the variability of the Quaternary 10 million years farther back into the Cenozoic Era in an even more dynamic scenario.

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Laminin β2 (S-Laminin): A New Player at the Synapse

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When the growth cone of a motor axon meets the surface of a myotube, the event locally transforms both cells (*1*). The motile growth cone becomes a mature nerve terminal, stably attached to the muscle cell and specialized for rapid, focal secretion of the neurotransmitter acetylcholine (ACh); the muscle cell surface accumulates receptors for ACh beneath the nerve terminal and concentrates the enzyme that metabolizes ACh, acetylcholinesterase, in the synaptic cleft. These specialized postsynaptic structures convert pulses of released ACh into brief electrical responses that trigger a muscle action potential and ultimately contraction. This complex synaptic structure induced by nerve-muscle contact is meant to last a lifetime: If either partner is damaged and replaced by regeneration, the remaining structures induce the appropriate specialization in the regenerating cell precisely at the original synaptic site.

The ability of a regenerating axon to find the site of the original synapse on a denervated muscle surface is remarkable; although the old synapse represents less than 0.1 percent of the muscle surface, virtually all new contacts form at that site. Over 15 years ago McMahan and his colleagues demonstrated that the synaptic site was marked by molecules in the synaptic basal lamina joining the two cells, and that these are sufficient to specify synaptic differentiation of either nerve or muscle during regeneration (*2*). The mechanism by which one of these markers, laminin β2, is localized to the synapse is described in a report by Sanes and his colleagues on p. 413 of this issue of *Science* (*3*).

Formation of the neuromuscular synapse, either during development or during regeneration, requires that dozens of macromolecules be concentrated at the synaptic site (*1*). These form a complex structure that extends from the cytoplasm of one cell across the synaptic gap into the cytoplasm of the other. Most of the synaptic components provided by each cell are made in the absence of the other cell; myotubes cultured without nerves, for example, make ACh receptors that spontaneously aggregate into clusters. These aneural clusters are associated with both cytoskeletal and basal lamina components that are normally part of the mature synaptic structure (*4*).

When a nerve contacts a muscle, one of the earliest steps in the assembly of the synaptic structure is the formation of a new cluster of ACh receptors at the site of contact. The synaptic receptor cluster is apparently induced at this site by agrin, a proteoglycan released by the nerve and incorporated into the synaptic basal lamina (*5*); agrin-induced ACh receptor clustering subsequently induces the accumulation of other postsynaptic components at the synapse (*6, 7*). Agrin thus appears to be a key to postynaptic differentiation. The recent experiments by Sanes, Merlie, and their colleagues (*3, 8, 9*) now focus on a second basal lamina component—the β2 chain of laminin—and examine its localization to the synapse and its role in presynaptic differentiation. An intriguing feature of their results is that the same discrete domain in the molecule appears both to localize the molecule to the synapse and to mediate its effect on nerve terminals.

Laminin β2 (or S-laminin) was originally identified with antibodies that recognize a synapse-specific component of the basal lamina (*10*); cloning revealed the protein to be homologous to the B1 (or β1) chain of laminin (*11*). Subsequent experiments showed that laminin at the synapse contains the β2 chain, presumably complexed with α and γ chains, whereas the β1 chain of laminin is confined to extrasynaptic basal lamina (*9, 12*). In cultures of the C2 mouse muscle cell line, the two chains have a distribution analogous to that found in adult muscle: The β1 chain is widely distributed on the surface of myotubes and in the extracellular matrix, whereas the β2 chain is concentrated near the ACh receptor clusters that spontaneously arise in aneural cultures. By making chimeras of the two homologous chains and analyzing their distribution in stable C2 transfectants, Sanes and his colleagues have identified a region of 16 amino acids near the carboxyl terminal of the β2 chain that is responsible for its association with ACh receptor clusters (*9*).

How is the β2 chain localized to the ACh receptor? Although the β2 chain appears on the surface only in association with ACh receptor clusters, it is made throughout the length of the transfected myotubes. In contrast to ACh receptors, which are free to diffuse in the membrane, the β2 chains, because they are part of the extracellular matrix, are unlikely to concentrate near ACh receptor clusters by lateral migration at the cell surface. Consistent with this idea, the accumulation of another synaptic basal lamina component, acetylcholinesterase, requires protein synthesis (*6*). Basal lamina containing β2 laminin chains must thus arise either by local intracellular incorporation of the β2 chains into laminin, by local transport of β2 laminin to the surface, or by local incorporation of β2 laminin into the basal lamina (either intracellularly or on the surface). Identification of the synap-

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