

REPORTS

tant role in locomotor propulsion than the forelimbs, which were probably important in food estimation. Because of this, the body mass estimation based on the femur is more reliable: *P. pattersoni* probably weighed ~700 kg. With *Phoberomys*, the size range of the order is increased and Rodentia becomes one of the mammalian orders with the widest size variation, second only to the Diprotodontia (kangaroos, koalas, wombats, and possums) (fig. S1).

The fossil record of Caviomorpha is extensive, with 140 fossil genera recognized in a recent review (8), but no form competes with *Phoberomys* in terms of size. *Artigasia magna* from the upper Pliocene of Uruguay is reported to be gigantic, but its lower teeth are only ~60% the size of those of *P. pattersoni* (19). *Artigasia*, like most fossil rodents, is known based only on dental and mandibular parts.

The paleoenvironment in which *P. pattersoni* was found and the associated fauna indicate that this rodent was either semiaquatic or foraged in or near water, as capybaras do. *P. pattersoni* had a deep and massive horizontal ramus of the mandible, correlated with a high degree of hypsodonty. *Phoberomys* clearly had an abrasive diet, perhaps consisting of seagrasses. Potential predators could have been the many crocodiles reported from Urumaco, including some of the largest forms that ever existed, such as *Purussaurus* spp. Contemporaries included *Stupendemys geographicus*, the world's largest turtle (20).

References and Notes

1. D. Starck, *Lehrbuch der Speziellen Zoologie. Wirbeltiere. Teil 5: Säugetiere* (Gustav Fischer Verlag, Jena, Germany, 1995).
2. J. J. Flynn, A. R. Wyss, *Trends Ecol. Evol.* **13**, 449 (1998).
3. R. F. Kay, R. H. Madden, R. L. Cifelli, J. J. Flynn, Eds., *Vertebrate Paleontology in the Neotropics. The Miocene Fauna of La Venta, Colombia* (Smithsonian Institution, Washington, DC, 1997).
4. *Léxico Estratigráfico de Venezuela* (Ministerio de Energía y Minas, Boletín de Geología, Caracas, ed. 3, 1997).
5. A. Mones, *Ameghiniana* **17**, 277 (1980).
6. A. Mones, *Comun. Paleontol. Mus. Hist. Nat. Montev.* **2**, 1 (1997).
7. F. R. Negri, J. Ferigolo, *Bolet. Mus. Pare. Emilio Goeldi* **11**, 1 (1999).
8. M. C. McKenna, S. K. Bell, *Classification of Mammals Above the Species Level* (Columbia Univ. Press, New York, 1997).
9. S. O. Landry, *Univ. Calif. Pub. Zool.* **56**, 1 (1957).
10. M. G. Vucetich, D. H. Verzi, J.-L. Hartenberger, C. R. Acad. Sci. Ser. II Sci. Terre Planetes **329**, 763 (1999).
11. L. Kraglievich, *An. Soc. Cient. Argent.* **114**, 155 (1932).
12. C. de Paula Couto, *Tratado de Paleomastozoología* (Academia Brasileira de Ciências, Rio de Janeiro, 1979).
13. B. Demes, W. L. Jungers, *Folia Primatol.* **52**, 58 (1989).
14. J. Bertram, A. A. Biewener, *J. Morphol.* **204**, 157 (1990).
15. A. R. Biknevičius, D. A. McFarlane, R. D. E. MacPhee, *Am. Mus. Novit.* **3079**, 1 (1993).
16. Predictive equations for body size (15): $\log W = \log a + b(\log AP)$, where W is body mass (in kilograms), $\log a$ is intercept, b is slope, and AP is the anteroposterior diameter of the bone examined. Anteroposterior proximal femur diameter (APF): $\log W =$

- $-1.678 + 2.518(1.80618)$, $W = 741.1$; anteroposterior distal humerus diameter (APH): $\log W = -1.467 + 2.484(1.6532)$, $W = 436.1$ kg.
17. A. R. Biknevičius, *J. Mammal.* **74**, 95 (1993).
18. The humerus/femur length ratio (H/F) and the (humerus + radius)/(femur + tibia) length ratio [(H + R)/(F + T)] in *P. pattersoni* (0.76 and 0.78, respectively) are average compared with those of other caviomorphs. For a sample of 17 extant caviomorphs, the mean values \pm SD were H/F = 0.80 ± 0.08 and (H + R)/(F + T) = 0.74 ± 0.09 . In the sample, there are no marked trends associated with growth or phylogeny (21). On the other hand, the ratios between femur versus humerus cross-sectional diameters (APF/APH) (15) show that the hindlimbs of *P. pattersoni* are robust. APF/APH is 1.42, whereas the average \pm SD for a sample of 19 caviomorph species (21) is 1.27 ± 0.18 . Robust hindlimbs in comparison to forelimbs characterizes also the clade composed of *Dinomys*, *Chinchilla*, and *Lagostomus* (mean \pm SD 1.40 ± 0.26), the closest relatives to *Phoberomys* among extant caviomorphs.
19. J. C. Francis, A. Mones, *Kraglieviana* **1**, 89 (1966).
20. R. C. Wood, *Breviora* **436**, 1 (1976).
21. M. R. Sánchez-Villagra, O. Aguilera, I. Horovitz, unpublished data.
22. R. M. Nowak, *Walker's Mammals of the World* (John Hopkins Univ. Press, Baltimore, ed. 6, 1999).
23. D. Huchon, J. P. Douzery, *Mol. Phylogenet. Evol.* **20**, 238 (2001).
24. We added *Lagostomus* to the tree to increase the relevant sampling in our scaffold analysis. All relevant treatments of caviomorph taxonomy and

phylogeny place *Lagostomus* together with *Chinchilla* (22).

25. M. S. Springer et al., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6241 (2001).
26. We thank J. Bocquentin, A. Ranci, A. Rincón, J. Reyes, D. Rodrigues de Aguilera, and R. Sánchez for help with fieldwork; J. Reyes and E. Weston for laboratory work; E. Weston and three anonymous reviewers for comments on the manuscript; O. Aguilera Jr. for assistance with digital imaging; S. Melendrez for reconstruction of the skeleton of *Phoberomys* in Fig. 2; D. Mörrike (Stuttgart) and E. Weber (Tübingen) for access to collections; and J. Bocquentin and A. Ranci for preliminary work on the identification of the giant rodent. Work in Venezuela by M.R.S.-V. was partially supported by the National Geographic Society and the University of Tübingen. The Smithsonian Tropical Research Institute and the Universidad Nacional Experimental Francisco de Miranda (UNEFM) supported the field and laboratory work of O.A. O.A. is a Research Associate of the Smithsonian Tropical Research Institute.

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www.sciencemag.org/cgi/content/full/301/5640/1708/DC1
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Amazonia 1492: Pristine Forest or Cultural Parkland?

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Archaeology and indigenous history of Native Amazonian peoples in the Upper Xingu region of Brazil reveal unexpectedly complex regional settlement patterns and large-scale transformations of local landscapes over the past millennium. Mapping and excavation of archaeological structures document pronounced human-induced alteration of the forest cover, particularly in relation to large, dense late-prehistoric settlements (circa 1200 to 1600 A.D.). The findings contribute to debates on human carrying capacity, population size and settlement patterns, anthropogenic impacts on the environment, and the importance of indigenous knowledge, as well as contributing to the pride of place of the native peoples in this part of the Amazon.

Was the Amazon a natural forest in 1492, sparsely populated and essentially pristine, as has been traditionally thought? Or, instead, were parts of it densely settled and better viewed as cultural forests, including large agricultural areas, open parklands, and working forests associated with large, regional polities (1–3). Despite growing popularity for the latter view (4–6), entrenched debates re-

garding pre-Columbian cultural and ecological variation in the region remain unresolved due to a lack of well-documented case studies (7, 8). Here, we present clear evidence of large, regional social formations [circa (c.) 1250 to 1600 A.D.] and their substantial influence on the landscape, where they have altered much of the local forest cover. Specifically, archaeological research in the Upper Xingu (Mato Grosso, Brazil), including detailed mapping and excavations of extensive earthen features (such as moats, roads, and bridges) in and around ancient settlements, reveals unexpectedly complex regional settlement patterns that created areas of acute forest alteration.

The Upper Xingu is unique in the southern peripheries of the Amazon as the largest contiguous tract of tropical forest still

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under indigenous resource management [Parque Indígena do Xingu (PIX)]. It remains little affected by 20th century mechanized development (Fig. 1). The Upper Xingu is a long-standing case study of indigenous Amazonian agriculture and ecology (9, 10) and one of the few places where contemporary observations about indigenous agriculture, land use, and settlement pattern can be systematically linked with archaeological and oral historical evidence (11). Long-term in situ cultural development of Xinguano peoples over more than 1000 years (12) is clearly documented by continuity in (i) utilitarian ceramics used to process and cook staple foods (13); (ii) settlement placement (at forest/wetland transitions) and local land use, marked by substantial forest and wetland alterations; and (iii) settlement form, notably circular plazas with radial roads (14) (movie S1).

In the Kuikuro study area (Fig. 2) (15), 19 major pre-Columbian settlements have been identified, generally separated by 3 to 5 km and linked by a system of broad, straight roads (16). Recognition and mapping of major earthworks at these sites reveal their articulation in a remarkably

elaborate regional plan (14). The earthworks include (i) excavated ditches in and around ancient settlements (up to 2.5 km long and 5 m deep); (ii) linear mounds or “curbs” positioned at the margins of major roads and circular plazas (averaging about 0.5 to 1.0 m in height); and (iii) a variety of wetland features, such as bridges, artificial river obstructions and ponds, raised causeways, canals, and other structures, many of which are still in use today. Similar constructed features (such as settlements, roads, weirs, and ponds) are known from culturally related peoples (principally Arawak speakers) across the southern Amazonian periphery (17–19) [supporting on-line material (SOM) text].

The integrated settlement configuration was in place by c. 1250 to 1400 A.D., based on radiocarbon dates from stratified deposits at X6, X11, and X13 (Table 1) (14, 20). Major curbed roads (10 to 50 m wide) articulate with plazas, ditches, and partition space within villages and across the broader landscape, notably linking settlements into “galactic” clusters across the region (21). The Ipatse cluster includes four major residential settlements (X6, X17, X18, and X22), linked to a fifth unfortified “hub” site (X13), with only limited residential occupation (Fig. 3A). Another cluster of sites, centered on X11, shows a similar pattern of a large (50 ha) fortified settlement connected to other smaller but still elaborate set-

tlements. In the case of the Kuhikugu (X11) cluster, the largest residential center is the hub (Fig. 3B). The primary nodes of each cluster are also linked by roads to smaller plaza settlements (fig. S1). On the basis of artifact and dark-earth distributions, it has been estimated that large sites, such as X6 and X11, had 15 to 25 ha of residential space, medium sites (X17, X18, X22) had 5 to 10 ha, and small sites had 2 to 5 ha (14). Thus, the actual residential area of a cluster like X6 or X11 was at least 40 to 80 ha in an area of about 400 km², with an estimated population of between 2500 and 5000 persons (or about 6 to 12.5 persons per km² in the study area).

Areas within each galactic cluster can be characterized as saturated anthropogenic landscapes, because virtually the entire area in and between major settlements, although not entirely cultural in origin, was carefully engineered and managed. Indeed, the road networks, oriented by the same system of cardinality that characterizes plaza spatial organization, partitioned the landscape into a gridlike or latticelike organization of nodes (plazas) and connecting thoroughfares, although patches and corridors of secondary and perhaps managed forests were likely common, as they are today. Mapped archaeological features correspond to patches of acutely modified secondary growth, distinctive from surrounding forest and easy to recognize in

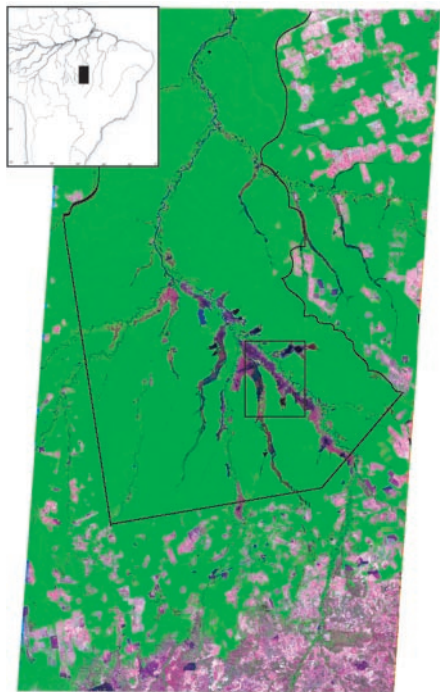


Fig. 1. Upper Xingu region image [Landsat 7 Enhanced Thematic Mapper, path 225, rows 68 to 69; 12 August 1999; image is a composite false-color infrared with bands assigned as 5-4-3 (red-green-blue) to provide the appearance of natural vegetation] with the approximate boundaries of the PIX and the Kuikuro study area (inset of Upper Xingu, Brazil). The ecological transition between the closed forests of southern Amazonia and the more open savanna/gallery forest of the Brazilian Planalto Central is shown (lower right).

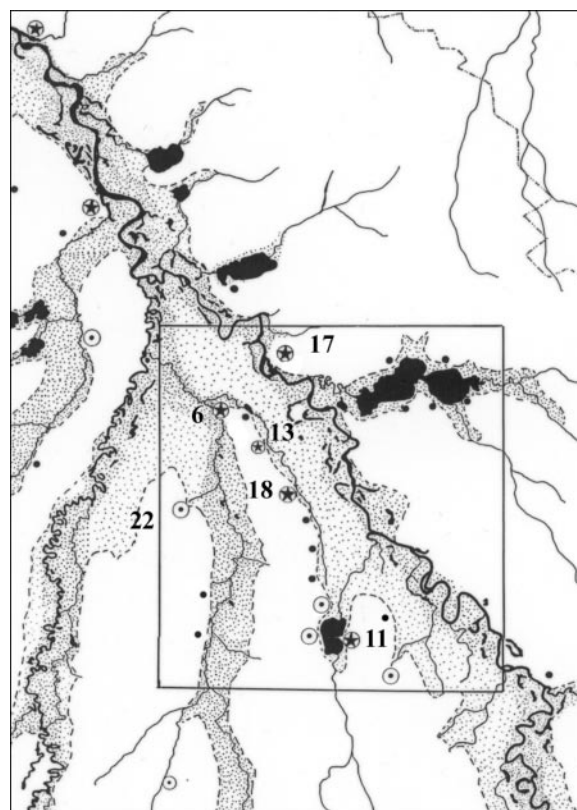


Fig. 2. Kuikuro study area showing the distribution of major ditched plaza centers (stars in circles), major plaza centers (open circles), and small plaza and non-plaza villages (black dots).

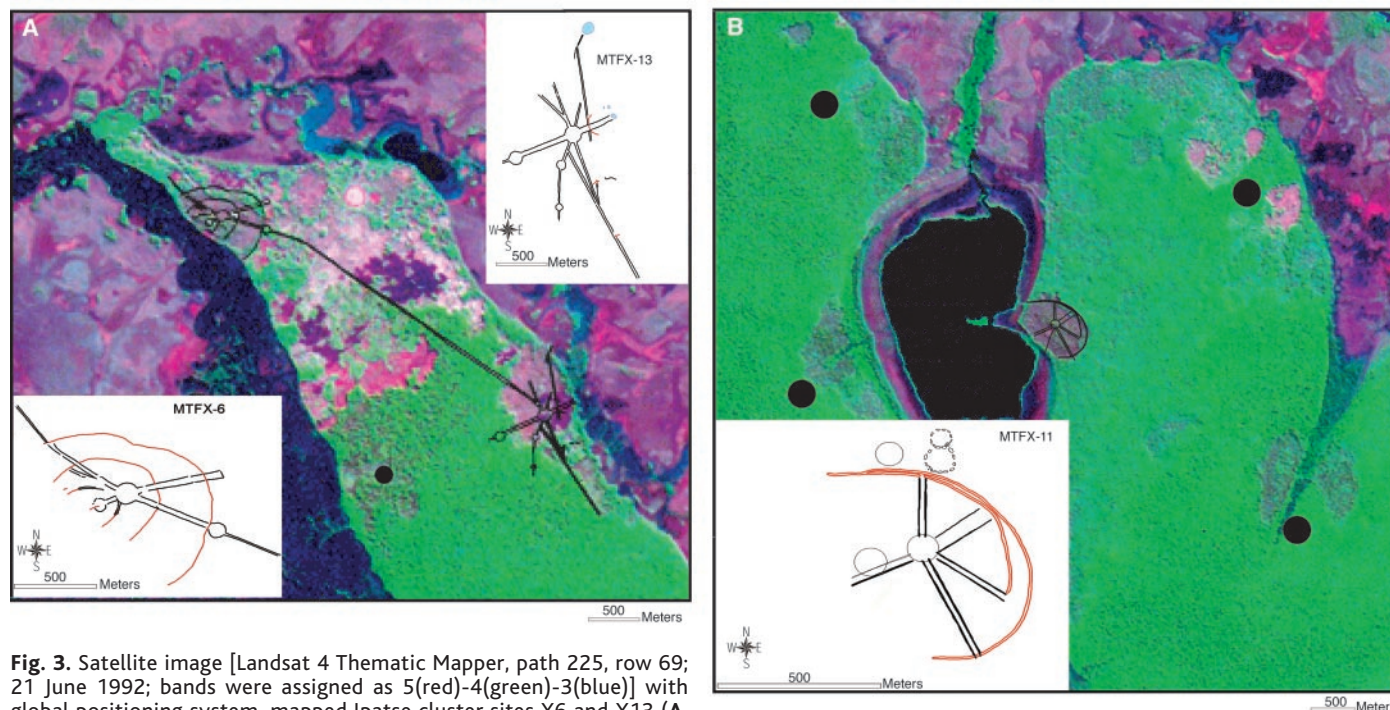


Fig. 3. Satellite image [Landsat 4 Thematic Mapper, path 225, row 69; 21 June 1992; bands were assigned as 5(red)-4(green)-3(blue)] with global positioning system–mapped Ipate cluster sites X6 and X13 (A, insets) linked by the “north-south road” and transit-mapped X11 (B, inset) linked by the “north-south road” and transit-mapped X11 (B, inset). Ditches are colored in red; road and plaza curbs are black. Fieldwork in 2003 demonstrates that roads extend fully from X13 to X18, and continue on to X19, X20, and beyond along the north-south

road, as well as across high ground to X17 and X22 (fig. S1); X11 roads also connect it to the four satellites. MTFX, Mato Grosso (the state), Formadores do Xingu (the archaeological region). The number refers to the site number.

Table 1. Radiocarbon dates from Nokugu (X6) and other sites in the Kuikuro study area, southern PIX. Beta 176135 to Beta 176144 are reported here for the first time. Calibrated age ranges for samples dated in 2003 (numbered 176135 and up) were reported by Beta-Analytic laboratory; previous (1994) dates were calibrated using CALIB 4.0 (29). ET, excavation trench of 1.0 by 10.0 m or more; EU, excavation unit of 1.0 m²; S, southern side of plaza or site; N, northern side; Ditch 1, outermost; Ditch 2, middle; Ditch 3, innermost ditch in all sites.

Lab no.	Site/unit	Conventional radiocarbon	2Σ-calibrated age range	Provenance
<i>Historical Xinguano (1700 A.D.–present)</i>				
Beta 176142	X6/ET2	20 ± 50	modern*	Ditch 3 (S), upper ditch infill
Beta 72260	X6/ET1	180 ± 60	1520–1940 A.D.	Ditch 2 (S), upper ditch infill
<i>Terminal “galactic” period (1400–1700 A.D.)</i>				
Beta 176137	X6/ET10	340 ± 60	1460–1640 A.D.	Ditch 2 (N), upper ditch infill
Beta 81301	X6/ET1	360 ± 70	1420–1640 A.D.	Ditch 2 (S), mid-ditch infill
Beta 176135	X6/ET3	440 ± 60	1420–1480 A.D.	Small plaza, suburb intact
Beta 72262	X11/EU1	440 ± 70	1400–1650 A.D.	North road, intact/curb interface
Beta 176140	X6/ET3	530 ± 60	1400–1430 A.D.	Small plaza, suburb intact
<i>Initial “galactic” period (1250–1400 A.D.)</i>				
Beta 176139†	X6/ET2	590 ± 60	1300–1420 A.D.	Ditch 3 (N), basal fill
Beta 177724†	X6/ET2	670 ± 60	1260–1410 A.D.	Ditch 3 (N), basal fill
Beta 88362	X13/EU1	690 ± 60	1260–1300 A.D.	Central plaza, suburb intact
Beta 78979	X6/ET1	700 ± 70	1230–1410 A.D.	Ditch 2 (S), sub-berm intact
Beta 176136	X6/ET4	710 ± 50	1270–1300 A.D.	Ditch 1 (S), basal fill
<i>Late developmental (900–1250 A.D.)</i>				
Beta 72263	X11/EU1	900 ± 60	1000–1250 A.D.	North road, suburb, basal intact
Beta 88363	X13/EU1	910 ± 80	1040–1250 A.D.	Central plaza, suburb base intact
Beta 72261	X6/ET1	1000 ± 70	950–1210 A.D.	Ditch 2 (S), sub-berm, base intact
Beta 176141	X6/ET5 1	030 ± 60	980–1030 A.D.	Central plaza, suburb base intact
<i>Initial Xinguano (pre-900 A.D.)</i>				
Beta 176143†	X6/ET2	1370 ± 60	640–690 A.D.	Mid-ditch 3 (N), mid-ditch
Beta 176138†	X6/ET10	2110 ± 40	190–60 B.C.	Ditch (N), basal fill

*Two additional modern dates, Beta 98978 and Beta 176144, are considered invalid. The former was redated with a sample slightly higher in profile (Beta 81301). †Beta 176139 is inverted with 176143 and was redated by 177724. Beta 176138 also comes from stratified but mixed context of ditch infill, and both likely represent earlier materials that are mixed in ditch construction.

satellite images. These patches or islands are identified in the indigenous knowledge systems, including diverse species whose distributions are generally restricted to anthrosols (dark earth)—called egepe by the Kuikuro—associated with ancient settlements (22–24) (SOM text). Some areas related to major pre-Columbian settlements, like X6, X11, and X13, have not returned to high forest after about 400 years of abandonment, although other areas (such as roads, hamlets, gardens, fields, and parklands) show a highly patchy forest regrowth typical of postabandonment (after 1600 to 1750) succession.

Recognition of the massive forest alterations associated with pre-Columbian occupations requires an understanding of local biodiversity in the context of the complex cultural history of the area. The composition of forest and wetland habitats reflects long-term cumulative changes, given that the settlement areas were occupied more or less continuously over many generations, as well as the large-scale alteration and management of local environments by dense late-prehistoric occupations (c. 1250 to 1650 A.D.). Present soil and biotic distributions, often isomorphic with the distribution of archaeological features, notably plazas, residential areas, roads, and roadside hamlets, are in large part the result of pre-Columbian land-management strategies. After c. 1600 to 1700, catastrophic

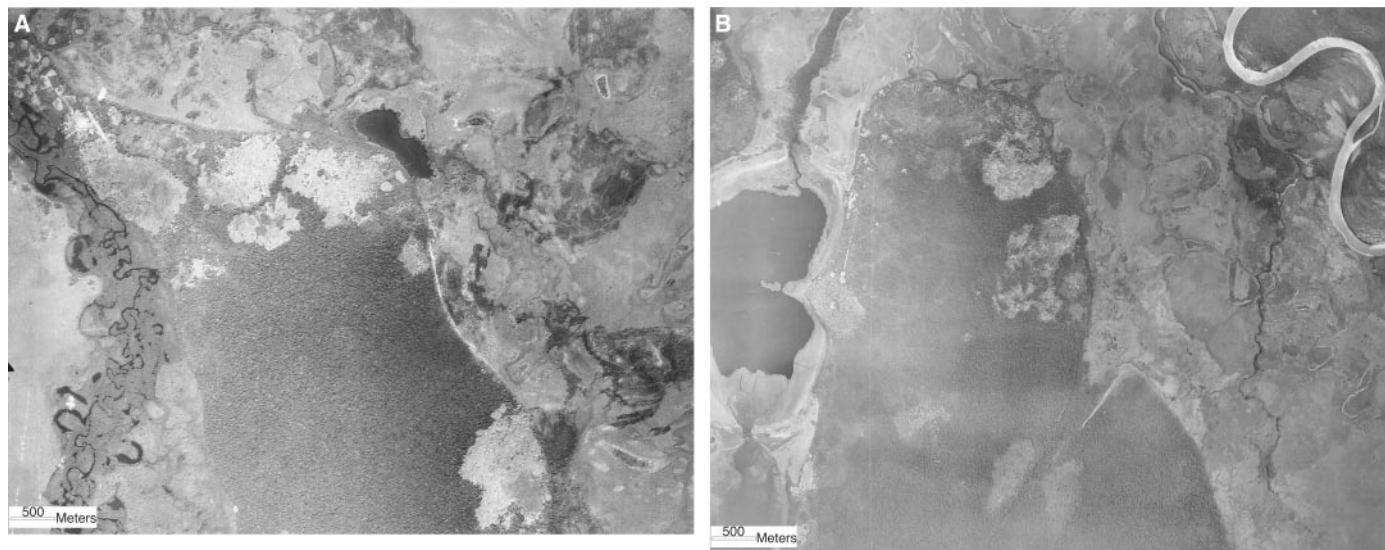


Fig. 4. Aerial photographs (U.S. Air Force/Força Aérea Brasileira 1967; 1:60,000) of Lake Ipatse (A) and Lake Kuhikugu (B) showing land-cover change during 6-year period (1961 to 1967) after abandonment (Kuhikugu) and reoccupation (Ipatse). Comparison with Fig. 3 (1992) of

same areas shows an ~30-year period. Graphic testimony of post-1492 decline in village size is shown by comparing the size of X11 with the four sequential Kuikuro villages (c. 1870s to 1961) in and north of the ancient site and by comparing X6 with the active village in 1967 and 1992.

depopulation (25) led to the abandonment of these works and many settlements, resulting in extensive reforestation in many areas. The scale of the prehistoric settlements, including exterior constructions, such as roads, hamlets, wetland structures, and cultivation areas, suggests that agricultural and parkland landscapes, rather than high forest, characterized the broad landscapes around ancient villages, as is true in contemporary villages. Metal technology, however, has increased the speed at which forests can be converted into mosaic parklands of dispersed manioc gardens, sapé grass fields, piqui groves, and secondary forests (26) (Fig. 4).

The Upper Xingu is a unique Amazonian example of a tropical forest way of life that supported large, densely settled, and integrated regional populations over the past 1000 years. Local ecology reflects the dynamic interaction between the natural environment, the influence of fairly large, settled human populations, and the legacy of Euro-American colonialism over the past ~500 years. Evidence of large, well-engineered public works (such as plazas, roads, moats, and bridges) in and between pre-Columbian settlements suggests a highly elaborate built environment, rivaling that of many contemporary complex societies of the Americas and elsewhere (SOM text). To suggest that Xinguano lands were intensively managed and developed, c. 1492, however, does not imply that indigenous land-use strategies, based on patchy development within long rotational cycles, are comparable to modern nonindigenous clear-cutting strategies (27, 28). Xinguano

cultivation and land management, indeed, provides a viable alternative. The present research emphasizes the critical importance of collaborative research strategies, including archaeological and ethnographic fieldwork, remote-sensed data analysis and geographic information systems, and most important, indigenous participation, to understand the complex interplay of ecological, historical, and political conditions in Amazonia before and after 1492.

References and Notes

- W. Balee, *Econ. Bot.* **7**, 1 (1989).
- W. Denevan, *Ann. Assoc. Am. Geog.* **82**, 369 (1992).
- D. Lathrap, *The Upper Amazon* (Praeger, London, 1970).
- C. Mann, *Science* **287**, 786 (2000).
- C. Mann, *Atlantic Monthly* **2001**, 41 (March 2002).
- This theory has been widely disseminated in popular media [e.g., "Fertile secret: How did ancient Amazonians turn sand into rich soil? Garbage," *American Broadcasting Corporation*, 19 September 2002, available online on <http://abcnews.go.com/sections/scitech/DyeHard/dyehard020919.html>; and "The secret of El Dorado," *British Broadcasting Corporation*, 19 December 2002, available online on www.bbc.co.uk/science/horizon/2002/eldorado.shtml].
- A. C. Roosevelt, *Am. Anthropol. Assoc. Pap.* **9** (1999), pp. 13–33.
- M. J. Heckenberger, J. B. Petersen, E. G. Neves, *Lat. Am. Antiq.* **10**, 353 (1999).
- R. Carneiro, *Subsistence and Social Structure* (Univ. of Michigan Press, Ann Arbor, 1957).
- R. Carneiro, in *Adaptive Responses of Native Amazonians*, R. Hames, W. Vickers, Eds. (Academic Press, New York, 1983), pp. 65–111.
- M. Heckenberger, *Antiquity* **72**, 633 (1998).
- Initial Xinguano occupations date sometime between 200 B.C. and 800 A.D., but the exact date is uncertain because the two earliest radiocarbon dates are from mixed deposits, and in one case (Beta-Analytic Laboratory number 176143) the dates are demonstrably out of sequence.
- Manioc, fish, and piqui fruit constitute 90% or more of the diet (9).
- Materials and methods are available as supporting material on *Science Online*.
- The Kuikuro study area is about the same as the Kuikuro (Carib Xinguano) traditional territory. It is about 1000 km² and is located in an area of traditional Xinguano occupations that is 25,000 to 35,000 km².
- Several additional large sites are known in the study area, on the basis of indigenous knowledge of dark-earth locations, and numerous smaller occupation sites are also known, including small road-side hamlets along the major roads.
- A. Metraux, *BAE, Bulletin 143* (Smithsonian Institution, Washington, DC, 1948), vol. 3, pp. 349–360.
- C. Erickson, *Nature* **408**, 190 (2000).
- M. Heckenberger, in *Comparative Arawak Histories: Rethinking Culture Area and Language Group*, J. Hill, F. Santos-Granero, Eds. (Univ. of Illinois Press, Urbana, 2001), pp. 99–122.
- Here, "galactic" describes the regional site clusters organized around a center or hub (X11 and X13), with several major residential sites connected to it.
- S. Tambiah, *Ritual, Performance, and Thought* (Harvard Univ. Press, Cambridge, MA, 1985).
- Dark earth forms in compost areas, although widespread burning produces minor but extensive soil alterations.
- W. I. Woods, J. McCann, *Yearb. Conf. Lat. Am. Geogr.* **25**, 7 (1999).
- J. B. Petersen, E. G. Neves, M. J. Heckenberger, in *The Unknown Amazon*, C. McEwan, E. Neves, C. Barreto, Eds. (British Museum Press, London, 2001), pp. 86–105.
- M. Heckenberger, in *Os Povos Indígenas do Alto Xingu: História e Cultura*, B. Franchetto, M. Heckenberger, Eds., [Editora Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, 2001], pp. 77–110.
- W. Denevan, *J. Steward Anthropol. Assoc.* **20**, 153 (1992).
- W. Denevan, *Cultivated Landscapes of Native Amazonia and the Andes* (Oxford Univ. Press, New York, 2001).
- N. Smith, A. Serrão, P. Alvim, I. Falesi, *Amazonia: Resiliency and Dynamism of a Land and Its People* (United Nations Press, Tokyo, 1995).
- M. Stuiver, G. W. Pearson, *Radiocarbon* **35**, 1 (1993).
- Ethnoarchaeological research in the Upper Xingu by M.J.H., conducted in collaboration with the Museu Nacional (UFRJ), the Museu Goeldi, and the

Kuikuro community, has been ongoing since 1991; 2001 to 2003 fieldwork was supported by NSF grant Behavioral and Cognitive Sciences no. 0004487 (2001 to 2003). We thank A. Melges, J. Petersen, and W. Hillman for their support.

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Resurrecting the Ancestral Steroid Receptor: Ancient Origin of Estrogen Signaling

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Receptors for sex and adrenal steroid hormones are absent from fully sequenced invertebrate genomes and have not been recovered from other invertebrates. Here we report the isolation of an estrogen receptor ortholog from the mollusk *Aplysia californica* and the reconstruction, synthesis, and experimental characterization of functional domains of the ancestral protein from which all extant steroid receptors (SRs) evolved. Our findings indicate that SRs are extremely ancient and widespread, having diversified from a primordial gene before the origin of bilaterally symmetric animals, and that this ancient receptor had estrogen receptor-like functionality. This gene was lost in the lineage leading to arthropods and nematodes and became independent of hormone regulation in the *Aplysia* lineage.

Vertebrate genomes contain six evolutionarily related nuclear receptors for steroid hormones: two for estrogens (ER α and ER β) and one each for androgens (AR), progesterins (PR), glucocorticoids (GR), and mineralocorticoids (MR). These ligand-activated transcription factors mediate the actions of hormones that direct sexual differentiation, reproduction, behavior, immunity, and stress response (1). There are no orthologs of these genes in the insect *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, or the urochordate *Ciona intestinalis* (2, 3), and a polymerase chain reaction (PCR) screen (4) failed to identify any of these genes outside the vertebrates. The most closely related nuclear receptors are the estrogen-related receptors (ERRs), an ortholog of which is present in the fruit fly genome (2). Based on this gene distribution, steroid receptors (SRs) are thought to have evolved in the chordate lineage some 400 to 500 million years ago, due to a duplication of a more ancient ERR gene (4–6). Estrogens and other vertebrate-type steroids appear to be involved in the reproductive endocrinology of certain mollusks, however (7, 8). Further, arthropods and nematodes are relatively closely related within the Ecdysozoan clade of molting organisms (9), suggesting the possibility

that the SR family may have been lost in the lineage leading to both phyla. A previous analysis of SR sequences indicated that the ancient progenitor of this protein class was most similar to extant ERs (10).

We used degenerate PCR and rapid amplification of cDNA ends to isolate an ER sequence (figs. S1 and S2) from a mollusk, the sea hare *Aplysia californica*. Using primers derived from vertebrate ERs, we amplified an ER-like cDNA sequence from both adult neural tissue and ovotestes of *A. californica* (11). The protein sequence of the *Aplysia* receptor's DNA-binding domain (DBD) is highly similar to that of the vertebrate ERs but much less similar to those of other nuclear receptors, including the ERRs (Fig. 1A). Within the DBD, the P

box, which mediates recognition of specific response elements by estrogen and other SRs (12), is identical only to that of the human ERs (Fig. 1B). The ligand-binding domain (LBD) of the *Aplysia* receptor is less conserved but is also most similar to that of the vertebrate ER. The *Aplysia* receptor's AF-2 activation domain—a small region in the LBD that mediates ligand-regulated interactions with coactivators (13)—is nearly identical to that of the human ERs but not to those of the ERRs or other SRs (Fig. 1B).

The true test of orthology is phylogeny, so we analyzed the relations among 74 steroid and related receptors, including the *Aplysia* ER, using maximum parsimony (MP) and Bayesian Markov Chain Monte Carlo (BMCMC) techniques (11). Both methods (Fig. 2A) strongly indicate that the *Aplysia* sequence is an ortholog of the vertebrate ERs. The node indicating orthology with the vertebrate ERs is well supported, with a BMCMC posterior probability of 100%, a bootstrap proportion of 90%, and a decay index of 6. Although BMCMC probabilities can sometimes overestimate statistical confidence (14), a 90% bootstrap normally indicates confidence well over 95% (15). Further, the maximum likelihood of this phylogeny is >100,000 times greater than that of the best phylogeny in which the *Aplysia* receptor is placed outside the clade of SRs. As this phylogeny shows, the gene duplication that produced the first SR preceded the ancient divergence of deuterostomes (the superphylum that includes chordates and echinoderms) from protostomes [mollusks, arthropods, nematodes, anne-

A	Percent similarity		B	Sequence alignment		
	DBD	LBD		P-box	AF-2	CTE
<i>Aplysia</i> ER	100	100	<i>Aplysia</i> ER	CEGCKA	DLLEMLDAHNS	(+27)
humanER α	88	35	humanER αL.....RL	(+46)
humanER β	85	32	humanER βL.....RL	(+30)
humanERR1	67	23	humanERR1	.A...	K.FL...E.MMD	*
humanERR2	70	30	humanERR2	.A...	K.FL...E.KV	*
humanERR3	67	30	humanERR3	.A...	K.FL...E.KV	*
<i>D.melan</i> ERR	67	25	<i>D.melan</i> ERR	.A...	K.FL...EPLAR	*
humanAR	58	21	humanAR	.GS..V	VDFFP..MAEIIIS	(+18)
humanPR	55	20	humanPR	.GS..V	VEFP..MSEVIA	(+19)
humanGR	58	21	humanGR	.GS..V	IEFP..LAEIIT	(+19)
humanMR	56	23	humanMR	.GS..V	VEFP..LVEIIS	(+19)

Fig. 1. The *Aplysia* ER protein sequence is most similar to that of human ERs. (A) Percent similarity of the *Aplysia* ER to steroid and related receptors of vertebrates and insects in the DNA- and ligand-binding domains. (B) Detail of sequences in the P box of the DBD, which mediates recognition of the core response element on DNA; in the AF-2 activation function of the ligand-binding domain, which is essential for ligand-activated transcription; and in the C-terminal extension (CTE) of the protein, with the length of the CTE in amino acids indicated. Dots show residues identical to those of *Aplysia* ER; an asterisk indicates the end of the coding sequence.

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