Oxygen isotopes of East Asian dinosaurs reveal exceptionally cold Early Cretaceous climates

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Early Cretaceous vertebrate assemblages from East Asia and particularly the Jehol Biota of northeastern China flourished during a period of highly debated climatic history. While the unique characters of these continental faunas have been the subject of various speculations about their biogeographic history, little attention has been paid to their possible climatic causes. Here we address this question using the oxygen isotope composition of apatite phosphate ($\delta^{18}O_p$) from various reptile remains recovered from China, Thailand, and Japan. $\delta^{18}O_p$ values indicate that cold terrestrial climates prevailed at least in this part of Asia during the Barremian—early Albian interval. Estimated mean air temperatures of about 10 ± 4 °C at midlatitudes (~42 °N) correspond to present day cool temperate climatic conditions. Such low temperatures are in agreement with previous reports of cold marine temperatures during this part of the Early Cretaceous, as well as with the widespread occurrence of the temperate fossil wood genus *Xenoxylon* and the absence of thermophilic reptiles such as crocodilians in northeastern China. The unique character of the Jehol Biota is thus not only the result of its evolutionary and biogeographical history but is also due to rather cold local climatic conditions linked to the paleolatitudinal position of northeastern China and global icehouse climates that prevailed during this part of the Early Cretaceous.

vertebrate phosphate | oxygen isotopes | paleoclimate

Since the last decade, continuous discoveries of Early Cretaceous invertebrates, plants and vertebrates in East Asia, and more particularly exceptionally preserved specimens in northeastern China belonging to the Jehol Biota, have fed numerous current evolutionary debates (1, 2). This latter assemblage is preserved in the lacustrine and volcanic sediments that mainly constitute the Yixian and Jiufotang formations of Liaoning Province. The peculiar character of the Jehol Biota, with "unusual" forms such as feathered dinosaurs, is clearly in part a result of the exceptional preservation of many fossils which show well preserved integumentary structures seldom preserved in other localities. However, it has been suggested that the Jehol Biota show peculiar floral and faunal compositions, which may, for instance, be indicative of a "relict" character (3) although this interpretation has been disputed (1). To date, possible relations between global climatic conditions and the taxonomic composition of the Jehol Biota have not been investigated, and no quantitative local climate reconstruction has been proposed so far. In the marine record, cold climatic intervals have been recognized during the Early Cretaceous period, with two major events occurring (*i*) during the early Valanginian, and (*ii*) from the late Barremian to the early Albian (4, 5). The most recent $^{40}Ar/^{39}Ar$ dating of sanidine crystals from tuff beds within the Yixian Formation and the base of the overlying Jiufotang Formation gave an age bracket of 129.7 ± 0.5 Ma to 122.1 ± 0.3 Ma for the deposition of the Yixian Formation (6). These ages correspond to a Barremian to early Aptian age interval which is encompassed by the second cold interval. We have estimated water $\delta^{18}O$ values and related mean air paleotemperatures at eight contemporaneous localities covering a large range of paleolatitudes in order to define a latitudinal climatic gradient and its influence upon the geographic distribution of East Asian fauna and flora.

Results and Discussion

We have used 99 new and 17 published [(7–9); Table S1] oxygen isotope compositions of apatite phosphate ($\delta^{18}O_p$) measured on dinosaurs, tritylodont synapsids, and freshwater crocodilian teeth, as well as turtles shell bones. These apatitic remains were recovered from deposits of the Yixian Formation and seven other Early Cretaceous formations in China, Japan, and Thailand. All these deposits are dated from the Barremian to the Aptian-Albian intervals and cover palaeolatitudes ranging from 21.0± 7.2 °N to 43.2 ± 8.0 °N (Table 1 and Fig. 1). Oxygen isotope compositions of apatite phosphates were measured using the procedure described in the *Analytical method* section, and are reported on in Table S1.

Preservation of the Original Oxygen Isotope Compositions. Secondary precipitation of apatite and isotopic exchange during microbially mediated reactions may alter the primary isotopic signal (10, 11). However, apatite crystals that make up tooth enamel are large and densely packed, and in the absence of high temperature conditions which Kolodny and others argue that they enable microbial mediated reactions to reset the bone phosphate oxygen isotopic signal, isotopic exchange might not affect the oxygen isotope composition of phosphates even at geological time scales (12, 13). Turtle shell and dinosaur bones should be more susceptible to diagenetic alteration because hydroxylapatite crystals

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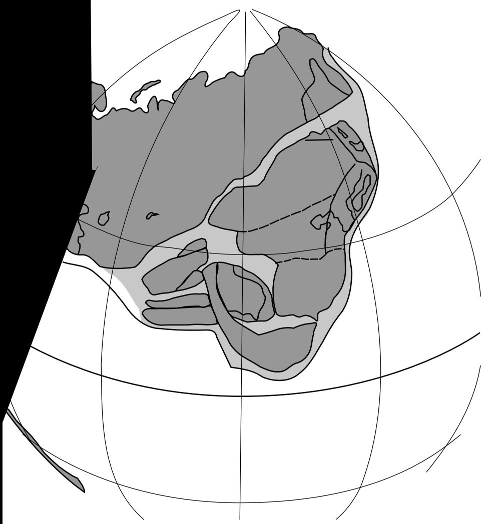
aller and less densely intergrown than thos
n though several case studies have shown
gen isotope composition can be preserve
remains (7–9, 15–17). Although no meth
onstrate definitely whether or not the ox
ion of fossil vertebrate phosphate was mod
ocesses, several ways to assess the preserva
nary isotopic record have been proposed [e.g.,
re the main argument supporting the preserva-
al oxygen isotope composition is the latitudinal
offset observed between the $\delta^{18}O_p$ values of
erms (dinosaurs and trytilodont synapsids) and
es and crocodilians) that mimics present day
offset observed between the $\delta^{18}O_p$ values of
ectotherms (Fig. 2). If early diagenetic processes
ey would have erased the expected offsets in
vertebrate remains having different ecologies
20).



(theropods) dinosaurs, systematic offsets in $\delta^{18}O_p$ values between these dinosaur groups that would reflect differences in diet, water strategies, and foraging micro habitats were expected (7, 22, 23). However, the $\delta^{18}O_p$ value differences observed between coexisting theropods, sauropods, ornithopods, ceratopsians, and ankylosaurs appear to be randomly distributed from one site to another (Fig. 2) instead of being ordered the same way at all sites. This observation suggests that these differences are more related to spatial or seasonal variability in ingested water $\delta^{18}O$ values than to taxon-specific ecological differences. Moreover, as dinosaur teeth took from a few months up to more than a year to grow depending on their size (24), significant differences in $\delta^{18}O_p$ values between teeth coming from the same deposit are expected. The same pattern can be observed on crocodilian and turtles $\delta^{18}O_p$ values but with less scattering due to their semiaquatic lifestyle and their living environment consisting of large water bodies (rivers, lakes) that buffer seasonal variations in local meteoric water $\delta^{18}O_{mw}$ values (Fig. 2). From present day data, it has

tation suggests that the distribution of choristoderes cannot be used as an indicator of warm climates simply on the basis of a supposed ecological analogy with crocodilians.

The relatively cold climatic conditions under which the formations containing the Jehol Biota were deposited may partly explain their singularity, notably by comparison with assemblages from other parts of Asia, which during the Early Cretaceous were located farther South. Such comparison applies, in particular, to the Barremian (?) Sao Khua and Aptian Khok Kruat formations of northeastern Thailand, which have yielded abundant vertebrate remains, and for which isotopic data are available (7). Although there are a few faunal elements shared by the Jehol Biota and the Khok Kruat Formation, such as the ceratopsian dinosaur *Psittacosaurus* (37), faunal similarities between the two formations seem to be very limited. Such differences may partly be owing to different depositional environments between the fluvial Thai formations and the largely lacustrine formations of northeastern China, with consequences in both composition and preservation. However, the different climatic conditions, themselves linked to geography, may have played a major role by preventing forms restricted to warm environments from entering northeastern Asia during part of the Early Cretaceous. More globally, the palaeotemperatures estimated from the oxygen isotope compositions of Jehol Biota fossils support previous claims that "icehouse" events took place during the Early Cretaceous, resulting in climatic conditions that might have been close to present day global climate but with a lesser extent of polar ice caps (5). The peculiar composition of the Jehol Biota may therefore largely reflect relatively cold climatic conditions in northeastern Asia during part of the Early Cretaceous, which contrasts with the usual "greenhouse" conditions under which many Mesozoic ecosystems flourished. A climatic gradient similar in some respects to the present one may at least partly explain the differences between the Jehol Biota and floral and faunal assemblages from regions located farther South.

Analytical method. Measurements of oxygen isotope compositions of apatite phosphate consist in isolating phosphate ions using acid dissolution and anion-exchange resin, according to a protocol derived from the original method published by Crowson et al. (38) and slightly modified by Lécuyer et al. (39). Silver phosphate was quantitatively precipitated in a thermostatic bath set at a temperature of 70 °C. After filtration, washing with double deionised water, and drying at 50 °C, 15 mg of Ag_3PO_4 were mixed with 0.8 mg of pure powder graphite. Oxygen isotope ratios were measured by reducing silver phosphate to CO_2 using graphite reagent (40, 41). Samples were weighed into tin reaction capsules and loaded into quartz tubes and degassed for 30 min at 80 °C under vacuum. Each sample was heated at 1,100 °C for 1 min to promote the redox reaction. The CO_2 produced was directly trapped in liquid nitrogen to avoid any kind of isotopic reaction with quartz at high temperature. CO_2 was then analyzed with a Thermo-Finnigan MAT253 mass spectrometer at the Institute of Geology and Geophysics, Chinese Academy of Sciences. Isotopic compositions are quoted in the standard δ notation relative to V-SMOW (Vienna Standard Mean Ocean Water). Silver phosphate precipitated from standard NBS120c (natural Miocene phosphorite from Florida) was repeatedly analyzed ($\delta^{18}O = 21.7 \pm 0.2‰$; $n = 37$) along with the silver phosphate samples derived from the fossil vertebrate remains.

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1. Zhou Z, Barrett PM, Hilton J (2003) An exceptionally preserved Lower Cretaceous ecosystem. *Nature* 421:807–814.
2. Barrett PM, Hilton JM (2006) The Jehol Biota (Lower Cretaceous, China): new discoveries and future prospects. *Integrative Zoology* 1:15–17.
3. Luo Z (1999) A refugium for relicts. *Nature* 400:23–25.
4. Pucéat E, et al. (2003) Thermal evolution of Cretaceous Tethyan marine waters inferred from oxygen isotope composition of fish tooth enamels. *Paleoceanography* 18:1029, doi: 10.1029/2002PA000823.
5. Price GD (1999) The evidence and implications of polar ice during the Mesozoic. *Earth Science Reviews* 48:183–210.
6. Chang S, Zhang H, Renne PR, Fang Y (2009) High-precision ^{40}

33. Markwick PJ (1998) Fossil crocodilians as indicators of Late Cretaceous and Cenozoic climates: implications for using palaeontological data in reconstructing palaeoclimate. *Palaeogeogr Palaeocl* 137:205–271.
34. Tarduno JA, et al. (1998) Evidence for extreme climatic warmth from Late Cretaceous Arctic vertebrates. *Science* 282:2241–2244.
35. Liu J (2004) A nearly complete skeleton of I *kechosaurus pijiagouensis* sp. nov.(Reptilia: Choristodera) from the Jiufotang Formation (Lower Cretaceous) of Liaoning, China. *Vertebrat Palasiatic* 42:120–129.
36. Zhou Z (2006) Evolutionary radiation of the Jehol Biota: chronological and ecological perspectives. *Geol J* 41:377–393.
37. Buffetaut E, Suteethorn V, Khansubha S (2007) in Proceedings of the International Conference on Geology of Thailand: towards sustainable development and sufficiency economy. (Department of Mineral Resources, Bangkok), pp 338–343.
38. Crowson RA, Showers WJ, Wright EK, Hoering TC (1991) A method for preparation of phosphate samples for oxygen isotope analysis. *Anal Chem* 63:2397–2400.
39. Lécuyer C, Grandjean P, O'Neil JR, Cappetta H, Martineau F (1993) Thermal excursions in the ocean at the Cretaceous-Tertiary boundary (northern Morocco): $\delta^{1837.}$ e svert1373.2(5is28)]TJ5nt.Tc()Tj2400.r Palaeocl