

Forest contraction in north equatorial Southeast Asia during the Last Glacial Period

Christopher M. Wurster^{a,1,2}, Michael I. Bird^b, Ian D. Bull^c, Frances Creed^c, Charlotte Bryant^d, Jennifer A. J. Dungait^{c,3}, and Victor Paz^e

^aSchool of Geography and Geosciences, University of St. Andrews, St. Andrews, Fife KY16 9AL, United Kingdom; ^bSchool of Earth and Environmental Sciences, James Cook University, Cairns, QLD 4870, Australia; ^cOrganic Geochemistry Unit, Bristol Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, United Kingdom; ^dNatural Environment Research Council, Radiocarbon Laboratory, East Kilbride G75 0QF, United Kingdom; and ^eArchaeological Studies Program, University of the Philippines, Diliman, Quezon City 1101, Philippines

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Today, insular Southeast Asia is important for both its remarkably rich biodiversity and globally significant roles in atmospheric and oceanic circulation. Despite the fundamental importance of environmental history for diversity and conservation, there is little primary evidence concerning the nature of vegetation in north equatorial Southeast Asia during the Last Glacial Period (LGP). As a result, even the general distribution of vegetation during the Last Glacial Maximum is debated. Here we show, using the stable carbon isotope composition of ancient cave guano profiles, that there was a substantial forest contraction during the LGP on both peninsular Malaysia and Palawan, while rainforest was maintained in northern Borneo. These results directly support rainforest “refugia” hypotheses and provide evidence that environmental barriers likely reduced genetic mixing between Borneo and Sumatra flora and fauna. Moreover, it sheds light on possible early human dispersal events.

biogeography | paleoecology | refugia | stable isotope | Sundaland

Equatorial Southeast Asia contains many unique endemics that contribute to the region's designation as a “biodiversity” hotspot, and understanding the region's environmental history bears directly on conservation issues (1–3). Its rich flora and fauna (20–25% of plant and animal species despite its small land area) is not solely a result of contemporary patterns in ecology and environment; perhaps no other region in the world bears such a strong imprint of historical environmental change on its present biogeography (3). Contemporary humid tropical conditions of insular Southeast Asia are maintained by the seasonal reversal of winds that bring the East Asian (northeast) and Australasian (southwest) monsoon systems. The shallow seas surrounding the submerged Sunda shelf are part of the Indo-Pacific Warm Pool (IPWP), among the warmest and wettest on Earth, playing several key roles in global atmospheric and oceanic circulation (4). The IPWP is an area where sea surface temperatures remain above 28 °C and precipitation excess is high due to monsoonal activity (4). As a result, it is globally important as a source of latent heat and moisture for global atmospheric circulation, and for its role in energy transfer between the Pacific and Indian oceans (5). Moreover, the region plays a key role in El Niño–Southern Oscillation dynamics (5).

During the Last Glacial Period (LGP; 125–10 kiloyear [kyr] ago), particularly the Last Glacial Maximum (LGM; 23–19 kyr ago), reduced global sea level exposed the continental shelf from south of Thailand to Sumatra, Java, and Borneo, revealing the contiguous continent Sundaland (6), with a land area the size of Europe. Oceanic temperatures surrounding Sundaland during the LGM were 2–3 °C cooler than today, and foraminiferal $\delta^{18}\text{O}$ values are interpreted to reflect reduced precipitation (5). Inferred environments on the exposed landmass are a contentious issue, particularly north of the equator, due to the paucity of well-dated proxy information from the LGM (6–8). It has been hypothesized that forest was replaced by savanna over

large areas (9, 10) or, alternatively, that lowland tropical rainforest persisted despite any reduction in rainfall (8, 11). In addition, model results for the region greatly vary, with some indicating that a broad continuous lowland tropical rainforest was maintained and others suggesting savanna over major portions of Sundaland during the LGM (6, 7). Surprisingly, there are no records from peninsular Malaysia and, in places where LGM sediments with suitable proxies are recovered, they may be compromised by biases such as riparian gallery forest vegetation and wind-blown tree pollen dominating riverine and offshore deposits (6, 8, 11, 12), or lowland swamp regions being biased toward indicating wetter conditions (13). Hence, even the general distribution of LGM vegetation is disputed.

An overlooked terrestrial depositional record exists in caves that serve as roosts to swiftlets (*Aerodramus* sp.) and/or insectivorous bats. Over time, their feces (guano) accumulate in deposits several meters thick, representing a time-transgressive proxy record amenable to radiocarbon dating (14). Fresh guano is composed dominantly of finely comminuted insect cuticles that are subsequently broken down by bacteria and fungi. Interactions with drip water, cave material, and guano lead to the formation of unique guano-specific phosphate and nitrogen minerals in an earthy organic matrix (15). These sediments contain multiproxy information from a variety of sources including the stable isotope composition of various extractable organic materials. Herein we use results obtained from $\delta^{13}\text{C}$ analyses of insect cuticles and molecular $\delta^{13}\text{C}$ analyses of normal alkanes (*n*-alkanes), both extracted from four guano deposits in northern Sundaland, to infer local vegetation changes during the LGP. Cavernicolous bats and birds feed within a limited area of the roost and are nonspecific in their predation of insects (16), which in turn are as abundant as their plant hosts (17). In lowland tropical locations, grasses use the C_4 photosynthetic pathway, whereas trees use the C_3 pathway. The different enzymatic pathways of fixing CO_2 result in $\delta^{13}\text{C}$ values of C_4 plants [−9 to −16‰ (per mille)] and their insect hosts that are substantially different from those of C_3 plants (−19 to −34‰) (18). This apparently large range in $\delta^{13}\text{C}$ values is considerably reduced at the biome level (19). Therefore, an integrated measure of the $\delta^{13}\text{C}$ values of insect carapaces will directly reflect the relative abundance of C_4 vegetation in a region (16), and variation in insect

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¹To whom correspondence should be addressed at the present address. E-mail: christopher.wurster@jcu.edu.au.

²Present address: School of Earth and Environmental Sciences, James Cook University, P.O. Box 6811, Cairns, QLD 4870, Australia.

³Present address: North Wyke Research, Okehampton, Devon EX20 2SB, United Kingdom.

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cuticular $\delta^{13}\text{C}$ values within a cave guano deposit will unambiguously reflect changes in the abundance of C_4 relative to C_3 vegetation in the region surrounding the cave (16, 20).

Insular Southeast Asia is rich in Karst terranes that house large populations of insectivorous bats and swiftlets. Although some cave sites are roosts to relatively large populations of fruit bats, we limited our selection to those with insectivorous colonies. Extraction of insect cuticles from the guano sediment, pH, and C:N ratios confirmed that insectivorous populations remained dominant throughout each record. We located four sites with LGM sediment deposition within $\sim 10^\circ$ north of the equator (Fig. 1). Along a transect from west to east are deposits in Batu cave ($3^\circ 13'\text{N}$, $101^\circ 42'\text{E}$) near Kuala Lumpur in peninsular Malaysia; Niah cave ($3^\circ 49'\text{N}$, $113^\circ 46'\text{E}$) in Sarawak, northern Borneo, and two sites in Palawan, Philippines, Gangub cave ($8^\circ 31'\text{N}$, $117^\circ 33'\text{E}$) in the south and Makangit cave ($10^\circ 28'\text{N}$, $119^\circ 27'\text{E}$) in the north. Age control is provided by radiocarbon dates on insect cuticles (14) taken from discrete intervals through each guano sequence, one charcoal sample from the Batu deposit, and three solvent-extracted bulk guano samples. Radiocarbon dates were calibrated to calendar years using the IntCal09 calibration curve (21) implemented using OXCAL 4.1 (22) (Tables S1, S2, and Fig. S1).

Results

The $\delta^{13}\text{C}$ profile from the Batu deposit indicates that C_4 biomass was a significant component of regional vegetation from at least 35 until 16 kyr ago, with values remaining above -22.6‰ until the end of the LGM (*SI Text* provides information on C_4 production estimates). After the LGM, an initial decline in $\delta^{13}\text{C}$ values occurred at ~ 14.7 kyr ago, with an increase in $\delta^{13}\text{C}$ values to -23.3‰ occurring between 13.4 and 12.5 kyr ago. Dominantly C_3 (forest) values (-26‰) are evident after 10.5 kyr ago and persisted until the present (Fig. 2). In the Niah deposit, Holocene sediment is missing due to mining of the upper part of the sequence for fertilizer, but $\delta^{13}\text{C}$ values of insect cuticles from ~ 50 – 10.7 kyr ago indicate that C_3 -dominant vegetation persisted through the LGM, with $\delta^{13}\text{C}$ values consistently between -24.7 and -26.2‰ , aside from a brief increase to -22.9‰ at ~ 13.4 kyr ago. Both records from Palawan show the clearest example of forest collapse during

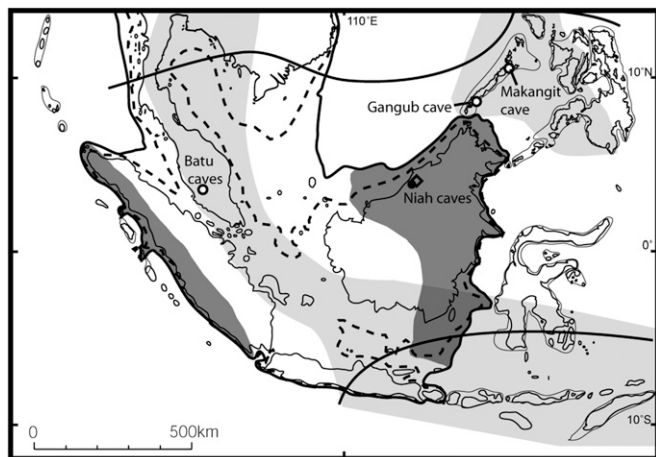


Fig. 1. Map of Southeast Asia showing the land-sea distribution during the Last Glacial Maximum estimated from the 120-m bathymetric line. A dashed line indicates the 50-m bathymetric line, which is a better representation of land-sea distribution at the time of human migration into the region. Study sites are displayed, where solid black (white) indicates evidence for forest (open) conditions during the LGM. The diamond represents the site location of a speleothem record (34). Earlier proposed savanna (9) and rainforest refugia (10) are indicated by light gray and dark gray shading. Solid lines indicate contemporary tropical lowland forest distribution.

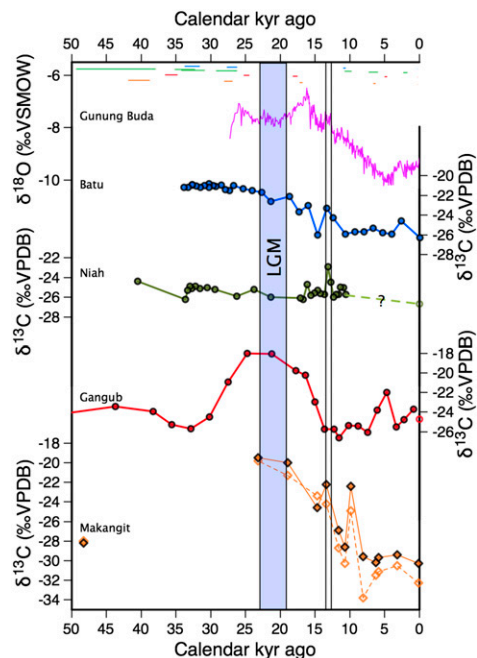


Fig. 2. $\delta^{13}\text{C}$ profiles of four guano deposits. We measured $\delta^{13}\text{C}$ values of insect cuticles for Batu (blue closed circles), Niah (green open circles), and Gangub (red closed circles). For the Makangit profile, lithogenic graphite contamination significantly affected results and could not be completely removed, so we measured $\delta^{13}\text{C}$ values of individual C_{29} (closed) and C_{31} (open) n -alkanes (orange diamonds). Although $\delta^{13}\text{C}$ values of n -alkanes are not directly analogous to those of insect cuticles, both are related to C_4 and C_3 relative abundances (*SI Text*). We also plot speleothem $\delta^{18}\text{O}$ values from Gunung Buda National Park (34), and mark the LGM and a period of reduced precipitation at 14.2 ± 0.2 kyr ago. For direct comparison, equivalent scales are used for $\delta^{13}\text{C}$ axes. Radiocarbon measurements are from insect cuticles, except for one charcoal sample and three solvent-extracted guano samples (Tables S1 and S2 and Fig. S1). VPDB, Vienna Pee Dee Belemnite; VSMOW, Vienna standard mean ocean water.

the LGM. Gangub cave has $\delta^{13}\text{C}$ values indicative of Pleistocene forest until ~ 33.5 kyr ago, after which time a substantial increase in $\delta^{13}\text{C}$ values occurred, from -26 to -18‰ at 21 kyr ago, indicative of open savanna (C_4) vegetation. Rainforest was again present in the cave area by 13.5 kyr ago. Makangit guano sediment contained lithogenic graphite, making it difficult to extract and analyze insect cuticles directly. To circumvent this problem, n -alkanes were extracted and compound-specific $\delta^{13}\text{C}$ values were determined (*SI Text* provides background information). Normal alkanes with a strong odd-over-even predominance represent epicuticular waxes, and are a direct biomarker of terrestrial vegetation (23, 24). As with the Gangub profile, the C_{29} and C_{31} n -alkanes exhibit relatively low $\delta^{13}\text{C}$ values suggesting rainforest at ~ 50 kyr ago, with highest values occurring during the LGM. The C_{29} n -alkane reaches maximum values of -19.5‰ beginning around 23 kyr ago. These high values declined after the LGM and by 8 kyr ago are -28.6‰ , showing a return of forest vegetation. By the mid-Holocene, $\delta^{13}\text{C}$ values are as low as -30.3‰ , and continued to be low until the present. Two increases in $\delta^{13}\text{C}$ values punctuated this overall decline, dated at 13.1 and 9.7 kyr ago. An analogous trend is also observed by plotting an n -alkane abundance ratio ($n\text{-C}_{29}/n\text{-C}_{31}$), the changes being derived directly from an input of epicuticular waxes from different plant species with time (23) (Fig. S2).

Discussion

Forest Contraction in Northern Sundaland. There are few well-dated LGM records from Sundaland, leading to the use of modern

biogeographic patterns and undated geomorphic evidence and even lack of evidence to interpret general vegetation distribution (6), much of which remains is conflicting (1). The southern portion has considerably more information than north of the equator. Taken together, there are either conflicting interpretations or complete lack of information for more areas than for those where there is substantial agreement. This study unequivocally demonstrates that savanna expanded in the Sundaland region north of the equator, by at least 400 km on peninsular Malaysia, and was an important contributor to plant biomass (Table S3, *SI Text*, and Fig. S3). A recent vegetation model for the region found that a continuous belt of lowland tropical rainforest should have persisted (7), and this conclusion is supported by pollen analysis of LGM river and offshore sediments from the South China Sea (8, 11). However, an increasing number of genetic studies show that migration between Sumatra and Borneo was extremely limited during the LGM even though the major islands were connected for most of the last 70 kyr (25–29), and possible rainforest refugia were inferred using termite morphology and taxonomic groupings (10), which worked well with other estimates derived from vicariant murine rodents (27). A savanna vegetation barrier such as we interpreted from the Batu $\delta^{13}\text{C}$ profile can explain such limited crossover of rainforest specialists between east and west Sundaland.

Extant rainforest-dependent species on Palawan today have been argued to suggest that forest persisted on the island during the LGM (12), whereas we infer open savanna conditions. Although a preliminary record from Palawan found savanna was present during the LGM on the north of the island (30), the interpretation of this record suffered from having only two radiocarbon dates and the presence of lithogenic graphite contamination. Nonetheless, some argued that the southern portion must have been forested and could have served as refugia (1, 7). We show using the guano profiles from Makangit and Gangub that both the north and south of Palawan were open savanna during the LGM and any rainforest refugia on the island must have been severely reduced. For example, this highly mountainous region may have provided refugia at higher elevation for forest specialists (31). It is likely that Palawan was not connected to Borneo during the LGM (32), and rainforest specialists would have derived from an earlier time. Other evidence from Niah cave suggests that forest cover was maintained in northern Borneo from at least 40 kyr ago (2, 33), and this is in agreement with our results.

Brief savanna re-expansions after the LGM apparent in guano $\delta^{13}\text{C}$ values in northern Palawan at 9.7 kyr ago and southern Palawan at 4.7 kyr ago may be due to local changes (e.g., human clearance), but increased $\delta^{13}\text{C}$ values occurred ~ 13.4 kyr ago at all locations except Gangub, suggesting a more regional climate-induced vegetation response at this time. A $\delta^{18}\text{O}$ profile from Mulu cave speleothems, Sarawak, indicates a drier period centered at 13.0 ± 0.2 kyr ago coincident with the Antarctic Cold Reversal, which interrupted an increasing trend in precipitation (34). Such a decrease in rainfall could be responsible for a short phase of forest contraction at this time, as indicated in the guano record.

Environments of Early Human Dispersal. Our results also provide fundamental evidence for understanding patterns of early human dispersal into the region. A major modern human expansion

occurred in Southeast Asia at ~ 60 – 40 kyr ago (35), possibly during a time of relatively mild climate and stable shorelines favoring coastal exploitation (36). We infer an environmental backdrop for Sundaland during the LGM that indicates a substantial area of savanna covered Sundaland north of the equator, potentially facilitating human dispersal through the region. This may have limited the area of tropical forest to be traversed to occupy Niah cave by 46 kyr ago (33). Although early humans could evidently deal with rainforest habitat at Niah, possibly with the aid of fire (33), dispersal over more familiar open woodland or savanna habitats through the core of Sundaland, followed by movement along the coast, provides an alternate scenario for human migration into Sundaland that does not require penetration of large areas of dense tropical rainforest.

Methods

All deposits were sampled from pits excavated through the accumulated guano. Exposed profiles were sampled at 3- to 5-cm intervals, adjusted where necessary to ensure that sample intervals did not cross stratigraphic boundaries. Samples were kept in a cold store at 4°C until freeze-dried. A detailed extraction procedure has been previously described for the recovery of insect cuticles from guano sediment (37). Approximately 250–300 μg of sample was placed into tin capsules and $\delta^{13}\text{C}$ values were measured using a Costech elemental analyzer fitted with a zero-blank autosampler coupled via a ConFloIII interfaced with a Thermo Finnigan DeltaPlus-XL isotope ratio mass spectrometer at the University of St Andrews Facility for Earth and Environmental Analysis. International Atomic Energy Agency-issued and internal reference materials were run alongside samples to normalize $\delta^{13}\text{C}$ values to the Vienna Pee Dee Belemnite (VPDB) reference scale and monitor instrument performance. All data are reported in per mille (‰) deviations from the VPDB-normalized reference standard scale. Reproducibility (SD) of three or four replicates of an internal laboratory reference material (processed from desert bat guano fertilizer) measured within each run of ~ 30 samples was within $\pm 0.2\%$.

Normal alkane fractions were extracted from guano, isolated, and then analyzed by gas chromatography (GC) (24). GC combustion isotope ratio mass spectrometry (GC/C/IRMS) analyses were made on 1.0 mL-aliquots using a Varian 3400 gas chromatograph fitted with a septum-equipped temperature programmable injector (SPI); the analytical column and temperature program used were the same as those used for the GC analyses. This was coupled to a Finnigan MAT Delta S stable isotope mass spectrometer.

Age models for each profile were constructed using radiocarbon measurements calibrated to calendar years using IntCal09 (21) implemented using OXCAL 4.1 (22). Calendar year as a function of depth was determined using point-to-point linear interpolation using a mean calendar age and associated 2σ confidence limit determined using the OXCAL program and based on the probability distribution of calibrated ages for a given radiocarbon age and depth midpoint. Sediment deposition rate is similar among deposits (Fig. S1). Extraction methods have been previously described (14, 37). Niah cave is missing Holocene sediment due to recent guano mining. Radiocarbon dates of extracted insect cuticles were prepared at the NERC Radiocarbon Facility and measured at the Scottish Universities Environmental Research Centre Accelerator Mass Spectrometry facility, East Kilbride, Scotland. In the deposit from Makangit, lithogenic graphite affected the ^{14}C result, necessitating correction before calibration (Table S2).

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