Tropical cloud forest climate variability and the demise of the Monteverde golden toad

Kevin J. Anchukaitis*1 and Michael N. Evans*1,4

*1Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964; 4Laboratory of Tree-Ring Research, University of Arizona, Tucson, AZ 85721; and 4Department of Geology & Earth System Science Interdisciplinary Center, University of Maryland, College Park, MD 20740

Edited by Lisa Graumlich, University of Arizona, Tucson, AZ, and accepted by the Editorial Board February 1, 2010 (received for review July 29, 2009)

Widespread amphibian extinctions in the mountains of the American tropics have been blamed on the interaction of anthropogenic climate change and a lethal pathogen. However, limited meteorological records make it difficult to conclude whether current climate conditions at these sites are actually exceptional in the context of natural variability. We use stable oxygen isotope measurements from trees without annual rings to reconstruct a century of hydroclimatology in the Monteverde Cloud Forest of Costa Rica. High-resolution measurements reveal coherent isotope cycles that provide annual chronological control and paleoclimate information. Climate variability is dominated by interannual variance in dry season moisture associated with El Niño Southern Oscillation events. There is no evidence of a trend associated with global warming. Rather, the extinction of the Monteverde golden toad (Bufo periglenes) appears to have coincided with an exceptionally dry interval caused by the 1986–1987 El Niño event.

The demise of the Monteverde golden toad (Bufo periglenes) in the montane cloud forest of Costa Rica in 1987–1988, as well as subsequent amphibian extinctions throughout the American tropics, has been believed to be a consequence of the interaction of global warming (1, 2) and the introduced chytrid fungus Batrachochytrium dendrobatidis (3–5). Analysis of the limited available weather data from Monteverde has suggested that a trend toward decreasing immersion cover since the late 1970s reflects the influence of increasing tropical air and sea surface temperatures (SST) (1, 2). Pounds and coauthors (1) argued that this pattern of increasing dry days implicated rising global temperatures due to anthropogenic greenhouse gas emissions. Nair et al. (6) and Lawton et al. (7) used a regional atmospheric model to demonstrate that deforestation in the tropical lowland forests upwind from the Monteverde Cloud Forest could also potentially result in elevated cloud base height and drier conditions.

Subsequent examination of a larger dataset of the last year of observation (LYO) of extinct neotropical amphibians in the genus Atelopus found a positive, lagged correlation between mean annual tropical (30°N to 30°S) temperatures and the timing of Atelopus extinctions (2). The authors hypothesized that temperatures at many montane locations in the Americas were shifting towards an “optimum range” for the growth of the chytrid fungus. This “chytrid-thermal-optimum hypothesis” suggested that environmental temperature trends associated with anthropogenic global warming were responsible for widespread amphibian extinctions due to chytridiomycosis.

This “climate-linked epidemic hypothesis” (2) postulates that global warming contributes to the development of optimal environmental conditions for the fungus. Upward trends in global temperatures could therefore already be fundamentally altering the suite of climatic conditions that maintain mountain forest ecosystems in Central America. General circulation model simulations of climate under doubled CO2 conditions predict higher lifting condensation levels and reduced cloud contact for tropical montane cloud forests as a result of increasing temperatures (8). Imprinted on the apparent instrumental drying trend at Monteverde, El Niño events also cause local increases in temperature and reductions in cloud cover and moisture (9). However, the role of climate change in neotropical anuran extinctions has been questioned on statistical grounds (10, 11). Without the context provided by long-term climate records, it is difficult to confidently conclude whether the extinction of the golden toad and other changes in cloud forest ecology are the consequence of anthropogenic climate forcing (2), land-surface feedbacks due to deforestation (6, 7), or natural variability in tropical climate (9).

Approach

We developed annual proxy records of climate variability from tropical montane cloud forest trees without annual rings at Monteverde (10.2°N, 85.35°W, 1500–1800 m) over the last century using stable isotopes (12, 13). The advantage of this approach is that it does not rely on the formation of yearly morphological growth hiatuses in trees, which in the tropics can often be absent (Fig. 1A), but rather takes advantage of the annual variation in the stable oxygen isotope ratio (δ18O) of water used by plants over the course of a year. This technique can also be applied where posited “growth rings” cannot be shown to be annual. Annual oxygen isotope cycles have been identified in lowland tropical forests (12, 14), and at Monteverde we have previously demonstrated that variation between the summer wet season and the cloud-dominated winter dry season is sufficient to induce an annual δ18O cycle along the radial xylem growth of cloud forest trees (13). The δ18O of cellulose in cloud forest trees reflects the seasonal change in the δ18O of source water as determined by the amount of rainfall and the 18O-enrichment of cloud water. On interannual time scales, departures from the mean annual cycle amplitude will result from anomalous rainfall and changes in temperature, relative humidity, and evapotranspiration (13, 15). At Monteverde, these are further related to changes in the intensity of moisture advection over the continental divide into the Pacific slope forests and the amount of cloud cover. Interannual changes in the annual δ18O cycle maxima can therefore be interpreted as moisture changes during the boreal winter dry season (approximately January to April).

We made high-resolution (200 μm increment) stable oxygen isotope ratio measurements along the radial growth axis of two Pouteria, mature canopy trees in the Sapotaceae family growing in the cloud forest on the Pacific slope below the continental divide at Monteverde, using a unique online induction pyrolysis system (16). The resulting δ18O data series have coherent annual oxygen isotope cycles we use for chronological control (Fig. 1 B and C) in the absence of rings. We developed an age model for both series by assigning the maxima of each discrete cycle to the...
Results
Annual $\delta^{18}$O cycles show a mean amplitude of 2.16‰ (Fig. 1 B–D; Fig. 2 A), with an average magnitude of year-to-year variability of 50% of this annual cycle ($\sigma = 1.08‰$). We observe significant correlations consistent with pilot studies and the interpretive hypothesis for annual and interannual variability described above. Collectively, dry and wet season temperature and precipitation, dry season zonal wind speed, and dry season relative humidity explain 40% of the annual cycle amplitude variability in our $\delta^{18}$O time series over the instrumental period at Monteverde. These empirical correlations are also consistent with expectations from independent, mechanistic modeling (15, 17) of expected cellulose $\delta^{18}$O as a function of climate (Fig. S1 and SI Text). Dry season amplitude anomalies also show a clear relationship to interannual climate variability in sea surface temperature anomalies (18) and zonal wind velocity (19) (Fig. 2 B and C). El Niño Southern Oscillation (ENSO) years in the $\delta^{18}$O chronology show a isotopic cycle enrichment 0.8‰ (greater than 1σ) above the mean (Fig. 2 A), with a strong mean positive anomaly of 2.0‰ for 1983, 1987, and 1998 alone (greater than 2σ above the mean). These years of discernible strong positive anomalies in the monthly $\delta^{18}$O chronology (Fig. 3 A) correspond to periods of dry season drought (greater number of consecutive dry days), negative dry season precipitation anomalies, and temperature anomalies of a degree Celsius or more (1). ENSO years have significantly higher $\delta^{18}$O values in the dry and early wet season (January through July, one-tailed paired t test, $p < 0.01$). In addition to dry season variability associated with these strong El Niño events, there are larger mean positive $\delta^{18}$O anomalies of ~1.8‰ in years of substantially warmer tropical Atlantic and Western Hemisphere warm pool (WHWP) temperatures (20). The anomalous peak (greater than 2σ above the mean) $\delta^{18}$O associated with warm WHWP events indicates a later onset of the rainy season and the effective moisture deficit persists for several months. The end of the dry season and early wet season (May through July) are significantly higher when WHWP events ($n = 4$) are considered alone (one-tailed paired t test, $p < 0.01$), consistent with the association of the largest magnitude WHWP warm events with El Niño events that have persistent boreal spring SST anomalies (20).

A weakening of local and equatorial Pacific 850 mb (~1, 500 m) easterly winds appears to consistently accompany the $\delta^{18}$O anomalies over the length of the reanalysis period (19) (Fig. 2 C). Those years with anomalously high maximum $\delta^{18}$O values occur simultaneously with dry season westerly zonal
...into a change in the elevation), shows an increase in average summer precipitation between 1960 and 1970 (21, 22). The previous spans the period of the transition, which shows an increase in the dry season amplitude, suggesting an overall damped season-and is related to a change in both the wet season minima and δ18O and likewise covary with ENSO and WHWP events. Anomalous midity display similar associations with the isotope chronology occurs in the middle or late 1960s (Fig. 1)

**Fig. 3.** Composite isotope time series and inferred moisture anomalies at Monteverde. (A) The composite monthly δ18O time series shows coherent annual isotope cycles back to 1900. The composite series was developed by averaging the two δ18O series after first diluting the variance of MV15 to that of MV12 as estimated over their common 253 month overlap. Differences in the long-term mean and common period variance of the two series are only slightly higher (0.35 and 0.41%) than analytical precision (0.28%). Gray shading indicates the full envelope of additive uncertainty due to analytical measurement precision and the observed differences in variance and mean between the overlapping section of the cores. Since these three different elements of total uncertainty are not independent, this envelope is likely conservative. δ18O enrichment associated with the 1986–1987 ENSO/WHWP event is highlighted, with horizontal dashed lines in (A) indicating the most likely range of dry season isotopic maxima for 1987, given uncertainties. Radiocarbon dates and their uncertainty are shown by filled triangles and their associated horizontal bars. (B) Monthly δ18O anomalies from the mean annual cycle emphasize that the time period associated with the 1986–1987 warm ENSO event was one of the driest of the last century. (C) Multidecadal variability in δ18O (SSA filtered, M = 20 years) shows changes in the magnitude of δ18O seasonality. (D) Deforestation rates in Costa Rica’s Atlantic forests upwind from Monteverde (30).

Wind anomalies. Because the slackening of the northeasterly tradewinds at Monteverde will influence moisture advection over the continental divide, the relationship between the dry season amplitude and local zonal wind anomalies from reanalysis data also accounts for the largest portion of the total variance in δ18O amplitude of any single local climate variable (r = 0.53, p = 0.01) (Fig. 2C). Higher temperature and lower relative humidity display similar associations with the isotope chronology and likewise covary with ENSO and WHWP events. Anomalous δ18O peaks that occur during warm ENSO and WHWP events coincide with increased Monteverde temperature, weaker zonal winds, and reduced relative humidity and precipitation; however, smaller δ18O dry season anomalies can also occur in ENSO neutral years when only one or two of these climatic factors is anomalous.

A distinct change in the amplitude of the annual δ18O cycle occurs in the middle or late 1960s (Fig. 1D; Fig. 3 A and C) and is related to a change in both the wet season minima and the dry season amplitude, suggesting an overall damped seasonality, reduced summer rainfall, and slightly increased dry season moisture for the period from the mid-1930s through the 1960s. There is support for this interpretation from the limited climate station data from the Pacific side of northwestern Costa Rica that spans the period of the transition, which shows an increase in precipitation between 1960 and 1970 (21, 22). The previous Monteverde station, downslope from the cloud forest (1,460 m elevation), shows an increase in average summer precipitation of approximately 200 mm over the 1960s, which could translate into a change in the δ18O of rainy season precipitation of as much as ~1.2‰ (13, 23), approximately the same as observed in our isotope chronology. However, the association between the δ18O time series amplitude and the concurrent zonal wind field retains its spatial pattern and magnitude, reinforcing our interpretation that the most consistent and proximal control on dry season moisture at Monteverde is the strength of the easterly winds. The change to high amplitude δ18O cycles in the late 1960s coincides with a shift in the sign of the summer North Atlantic Oscillation (NAO) index (24), an increase in interannual band (ENSO) variance in the tropical Pacific (25–27), a change in the seasonality of ENSO (28), and with other rapid changes in patterns of global atmosphere circulation, particularly in the tropical and Atlantic regions (24). Positive phases of the NAO, such as observed after the 1960s, are associated with positive precipitation anomalies in the early wet season in Central America (29). At the same time, deforestation rates in the Atlantic coast forests of Costa Rica increased (Fig. 3D) (30) and could have contributed to mesoscale alterations of boundary layer interactions sufficient to influence downwind dry season cloud formation (6, 7).

We empirically identify temporal patterns in our δ18O time series using singular spectrum analysis (SSA) (31–33). The identification of interpreted spectral components is robust to the choice of embedding dimension. Leading modes contain annual and 2–6 yr periodicities and explain 34% and 12% of total monthly signal variance, respectively. ENSO band frequencies contain 34% of the power at annual resolution. Only one significant low order eigenvector is dominated by multidecadal variance, with a prominent spectral peak between 20 and 30 yr (Fig. 3C). This reflects changes in the annual isotope amplitude, or seasonality, since we determined that removing the annual cycle prior to SSA causes this component to disappear entirely. A monthly anomaly series (Fig. 3B) is recovered by calculating the difference between each monthly δ18O value and the overall mean (n = 102) δ18O “climatolo” for the corresponding month.

**Discussion**

**Causes of the Demise of the Monteverde Golden Toad.** The early study of the relationship between the hydroclimatology of Monteverde and ENSO events by Pounds and Crump (9) noted the strong drying which occurred during the 1986–1987 El Niño event and identified higher temperatures and lower seasonal rainfall as well during the prior (1982–1983) El Niño. In that case, warmer Pacific SSTs were interpreted to correspond to warmer, drier conditions in the cloud forest. Based on statistical hypothesis testing, Pounds and coauthors (34) subsequently concluded that the abnormal dry conditions of 1987 were likely to have caused the multispecies population crash, which included the extinction of the Monteverde Golden Toad. Both of these papers speculated at the time that disease may have been the proximate cause of the extinction of the golden toad (9, 34). Chytridiomycosis, caused by the chytrid fungus _Batrachochytrium dendrobatidis_, was eventually identified as a major cause of amphibian extinction throughout the Americas, Europe, Australia, and New Zealand (3–5).

The “chytrid-thermal-optimum hypothesis” suggested that tropical temperature trends associated with anthropogenic global warming were responsible for widespread amphibian extinctions due to chytridiomycosis (2), and the earlier work (1) associated the extinction of the golden toad with dry conditions at Monteverde. Lips et al. (11) tested the robustness of the statistical comparison between tropical temperatures and _Atelespus_ LYO data, concluding that uncertainties in the choice of anuran observational data were sufficient to call into question the relationship. These authors instead sought to demonstrate that spatial patterns of extinction due to chytridiomycosis could be related to the spread of the fungus from a few centers of initial infection. Rohr et al. (2008) (10) questioned both conclusions and stated that “Almost all of our findings are contrary to the predictions of the ‘chytrid-thermal-optimum hypothesis.’” They also found that
there was no stable linear relationship between the diurnal temperature range and modeled growth of the chytrid fungus. Significant correlations were found, however, between modeled chytrid fungus mortality and decreases in cloud cover (10).

The significant drying trend in the number of days without rainfall identified by Pounds and coauthors (1, 2) is a result of the rapid increase in the number of annual dry days beginning with the strong 1982–1983 El Niño and the occurrence of long periods of drought during the 1998 dry season, also associated with ENSO. This trend is sensitive to the range of years and the threshold of consecutive days utilized (see Fig. S2 and SI Text). We have also determined that, due to the limited length of the record, there is no statistically significant difference \( p \geq 0.26 (35) \) in the correlation between the LYO for Atelopus and annual tropical temperatures \( (r = 0.76, p = 0.001) \), the Atlantic Multidecadal Oscillation index \( (r = 0.54, p = 0.04) \) (18, 36), or average spring-summer sea surface temperatures in the WHWP \( (r = 0.48, p = 0.06) \) (20).

Contrary to interpretations of the short instrumental record (1), no long-term trend in dry season hydroclimatological data is evident from our \( \delta^{18} \)O time series at Monteverde (1900–2002, \( p = 0.50; 1973–2000, p = 0.31 \)). Rather, variability at the interannual scale dominates the isotope signal, particularly during the period of increased ENSO variance since the late 1960s. The years 1983, 1987, and 1998 are the three driest winters in the last three decades (Fig. 3A). Collectively, the period from 1986 to 1988, associated with a strong El Niño event and an extensive WHWP that persisted into boreal late spring-summer, appears to be one of the driest in the last three decades and is amongst the driest of the last century (Fig. 3B). The strong warm ENSO event of 1997–1998 was also followed by demographic shifts in anuran populations at Monteverde (1). There is currently no consensus on how anthropogenic climate change will influence the El Niño Southern Oscillation (37, 38) and ENSO anomalies in the most recent decades (39). This suggests that the cause of the specific and well-documented extinction of the Monteverde golden toad was the combination of the abnormally strong ENSO-forced dryness (9) and the lethality (3) of the introduced chytrid fungus, but was not directly mediated by anthropogenic temperature trends, a finding from paleoclimatological data that is in agreement with statistical reconstructions (10, 11) of the “climate-linked epidemic hypothesis.”

In light of these results, the early hypotheses by Pounds and coauthors about the potential role of warm ENSO events in driving recent ecological change at Monteverde now appear prescient (9, 34). Our \( \delta^{18} \)O chronology from Monteverde allows us to conclude that one of the longest driest periods in the last 100 yr occurred during the 1986–1987 El Niño. It is possible that Batrachochytrium dendrobatidis was already present in Monteverde prior to that year (40). Dry conditions during the reproductive season in 1987 would have caused amphibian populations to coalesce around a few remaining wet microhabitats (1, 34, 41), where the fungus would have been able to spread rapidly through the population and ultimately result in extinction.

Sources of Uncertainty. While high-resolution oxygen isotope measurements allow us to resolve the annual cycle of source water \( \delta^{18} \)O and isotopic enrichment in cloud forest trees, and to therefore establish chronological control even in the absence of annual rings, there are a number of uncertainties that necessitate cautious interpretation of the chronological and climatic information in our \( \delta^{18} \)O time series. Our approach to developing records of past climate from tropical trees (12) has a closer methodological and procedural affinity to paleoclimate analysis using speleothems or corals than to classical dendrochronology. Although chronological uncertainty in this study is minimized through the combination of the identification of annual stable isotope cycles (13) and radiometric dating, we do not achieve the age model precision available from traditional approaches to dendrochronology that use large sample sizes and robust statistical pattern matching (42), nor can we be certain we have identified “missing” years. While the \( \delta^{18} \)O signal of cellulose from tree rings will be dominated by the enrichment due to transpiration and the source water signal associated with the amount effect (43), the latter accounts for only a portion of the total variability in precipitation \( \delta^{18} \)O (13) and the former could be influenced by several additional environmental and physiological factors; only the effects of precipitation, temperature, humidity, and wind are accounted for directly in our analysis. We have found that the observed annual and interannual \( \delta^{18} \)O variability in our Pouteria trees match those expected from both our hypothesis and mechanistic simulations. However, additional replication is necessary to place more confidence in estimates of multidecadal variability.

On the Role of Climate and Disease in Extinction Events. Epidemic-driven extinction may be the result of a series of biotic and abiotic interactions across spatial and temporal scales (11, 44). Drought or pluvial conditions associated with strong ENSO events are known to be associated with localized extinction (45, 46). In Yellowstone National Park, amphibian declines have been linked to the combination of decreasing precipitation, increasing temperatures, and wetland desiccation, which affects populations by contributing to increasing mortality, decreased migration, and reduced opportunities for wetland colonization (47). The decline of amphibians in El Yunque Forest in Puerto Rico is believed to be a consequence of a change in their behavior during dry periods, with populations moving from a dispersed distribution to a few protected microsites on the landscape, increasing their vulnerability to contagion (48). Similar patterns were observed at Monteverde prior to the multispecies population crash and extinction of the golden toad in 1987 (1, 9, 34, 41). Extinction events are an omnipresent feature of the history of life (49). Hence, while climate anomalies are not a feature of all extinctions (11, 44), it is unreasonable to expect that natural climate variability can interact with species life history and ecological community and population dynamics to contribute to extinctions, even as anthropogenic climate change continues to develop. Indeed, future increases in tropical temperatures, in conjunction with interannual or decadal variability, may exacerbate stresses on tropical montane ecosystems.

Materials and Methods

Stable Oxygen isotopes. Increment cores from two individual mature Sapotaceae (Pouteria) were collected at an elevation of 1,580 (MV12) and 1,580 m (MV15). MV12 was struck by lightning and subsequently felled in 2002. Radiocarbon measurements on MV15 revealed that it had an outer wood date more recent than 1960. The cores were subsampled in the laboratory at continuous 200 μm increments using a rotary microtome. The raw wood samples were chemically processed to α-cellulose using the Brendel method (50, 51) as modified for high-resolution sampling (12), and 300–350 μg of α-cellulose were wrapped in silver capsules and converted online to CO in a Costech High Temperature Generator/Elemental Combustion System (HTG/ECS) (16). At the time these analyses were conducted, this was the minimum sample size necessary to achieve acceptable measurement precision, and therefore determined the sampling increment on the 5 mm diameter cores collected from our Pouteria trees from Monteverde. The HTG uses a 1 MHz radio frequency induction heater to rapidly raise the temperature of a thin molybdenum crucible susceptor packed with graphite to >1,500 °C for highly efficient pyrolysis (16). Isotopic measurements were made using a Costech induction heater coupled to a ThermoFinnigan Delta XP continuous flow isotope ratio mass spectrometer (16). Measurement precision on a total of 320 interspersed Sigma Alpha Cellulose solid standards was 0.28‰.

Radiocarbon and Chronology Validation. Seven samples from the two cores were selected for radiocarbon analysis to test the \( \delta^{18} \)O-based age model. We estimate that maximum age model error for the earliest part of our chronology to be \pm 1 yr, based on the range of realistic age models as constrained by the 2σ resolution of the post-1955 radiocarbon dates. The older (1900–1949) portion of the chronology has an estimated maximum
50. Brerol D, Iannetta PPM, Stewart D (2000) A rapid and simple method to isolate pure δ13C, a span of 1900–1969 for MV15 has the largest cross-correlation with MV12 for the monthly δ18O values (r = 0.43, p = 0.016, [S5]), the monthly mean (r = 0.44, p < 0.01) and the monthly minimum (r = 0.65, p < 0.01) δ18O, which mirrors the independent high-resolution radiocarbon dating described above.

ACKNOWLEDGMENTS. Todd Lange was instrumental in securing radiocarbon dates. We are grateful for comments and suggestions from Julio Betancourt, Malcolm Ahyes, Mary Rack, Julie, Lan, Jonathan Overpeck, and Tim Shanahan. We benefitted tremendously from excellent laboratory and field assistance from John Buchanan, Mau-Chuang Foo, Lisa Wade, Frank Joyce, Alan Pounds, Rex Adams, Jim Burns, Sarah White, Arturo Cruz, Eladio Cruz, and Koki Porras. We thank the Organization for Tropical Studies for help with permits and Alan Pounds and the Tropical Science Center for access to the Monteverde Cloud Forest Reserve (Rafael Bolaños and Carlos Hernandez) and surrounding areas. We thank two anonymous reviewers for comments that helped improve the manuscript. This research was supported by a graduate fellowship from the National Science Foundation (NSF IGERT Program (DGE-0221594) (to K.J.A.), a Graduate Research Environmental Fellowship (to K.J.A.) from the U.S. Department of Energy, and NSF Grants ATM-0349356 and ATM-0321348 (to M.N.E.). Data are available from the NOAA World Data Center for Paleoclimatology (http://www.ncdc.noaa.gov/paleo/palo.html). This is UDEO Contribution #7226.
Supporting Information

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SI Text

Site Description. The Monteverde Cloud Forest sits astride the continental divide in the Cordillera de Tilaran in northwestern Costa Rica. Above 1,500 m elevation, vegetation is classified montane wet cloud forest (1) and its hydroclimatology is characterized by persistent immersion in the orographic cloud bank formed as the northeasterly trade winds moving across the warm waters of the Caribbean are forced to rise over the cordillera. Higher relative humidity, increased moisture, persistent cloud cover, and reduced temperatures support montane forests rich in endemic organisms and important for local hydrology (2, 3). During boreal summer (May to October), cloud forests receive most of their rainfall as the northward movement of the Intertropical Convergence Zone (ITCZ) brings convective storms to the region. During the dry season (approximately January through April), moisture advection and cloud water deposition are an important component of cloud forest hydrology (4), particularly on the leeward Pacific slope that would otherwise experience the effects of a pronounced dry season.

Stable Isotope Analysis and Interpretation. Following standard convention, oxygen isotope ratios are referenced and reported relative to the international Vienna Standard Mean Ocean Water:

$$\delta^{18}O = \left( \frac{^{18}O_{\text{Sample}} - ^{18}O_{\text{Ref}}}{^{18}O_{\text{Ref}}} \right) \times 1,000. \quad [S1]$$

Raw isotope ratio data were corrected based on the value of the solid standard material and measurements of monitoring gas (CO) with a known isotopic composition prior to and after every sample. Data quality assurance was assessed based on sample peak voltages, peak shape, background stability, and monitoring gas measurements.

The $\delta^{18}O$ time series from MV15 shows an abrupt change in growth rates at approximately 121 mm depth in the core. Accompanying this change is a substantial increase in the amplitude of the final eight annual oxygen isotope cycles. The rapid growth rates which correspond with the inner portion of the core result in an increase in the number of individual samples per annual cycle. The higher sampling rate could potentially translate into larger amplitude $\delta^{18}O$ cycles simply by virtue of more precisely resolving in time short-term changes in water use in the tree. Because this could possibly bias our climatic interpretation of the oxygen isotope time series for this earliest part of our record, for the purpose of climate analysis we statistically downsampled the sampling resolution to 6-10 samples per cycle to simulate that of the outer portion of MV15. Despite this a priori correction, however, the inner part of MV15 still has larger amplitude cycles than much of the outer core, and therefore we interpret this earliest decade cautiously, as these years could still contain a growth rate sampling bias.

Thus far, relatively little information exists about potential "juvenile effects" in oxygen isotope chronologies from trees. Treydte et al. (2006) (5) found that when trees were younger, the $\delta^{18}O$ of their xylem cellulose was enriched over that of older trees growing simultaneously. In the case of MV12 and MV15, we do not see any evidence of an enriched mean $\delta^{18}O$ in the earliest parts of the cores, and there is no indication that our cores reached pith. It cannot be ruled out that the increased variance in MV15 in the earliest part of the chronology, as discussed above, could be related to a juvenile effect, where younger trees with less extensive root systems use predominantly very shallow ground water; however, our conclusions do not depend on the interpretation of this part of the record. Much interesting work remains to be done to understand how trees might reflect age-related effects in the $\delta^{18}O$ of their xylem cellulose.

Heuristic and Mechanistic Model. The origin of annual cycles in the stable oxygen isotope composition of the wood of tropical cloud forest trees is the differential $\delta^{18}O$ composition of dry and wet season moisture (6, 7). This hypothesis is based on the assumption that the cloud forest receives moisture advection and cloud water from the Caribbean. However, water vapor from the Caribbean is not the only source of moisture for trees, particularly in the dry season. Cloud water has an enriched isotopic signature similar to tropical dry season rainfall (11, 13, 14, 15, 16). Dry season sources of moisture (the limited amount of rainfall and horizontal precipitation from orographic clouds) provide trees with sufficient water to avoid water stress and potentially the need for a seasonal growth hiatus, but have a distinct isotopic signature.

In order to develop objective, biochemical hypotheses for the mechanistic interpretation of cellulose $\delta^{18}O$ in tropical cloud forest trees, we used meteorological (17) and stable isotope data (11, 12) from Monteverde to simulate the sensitivity of $\delta^{18}O$ of cellulose in cloud forest trees to climate anomalies. The major obstacle to this exercise is the lack of any relative humidity or wind data or cloud observations from within the cloud forest itself. Anchukaitis et al. (2008) (7) were able to successfully simulate annual isotope cycles and their interannual variability in trees from below the main cloud forest elevations using the temperature and precipitation data from the Campbell weather station (17) and the relative humidity data modeled from a short period of meteorological observations in the premontane forest downslope from the Cloud Forest Reserve (11). For the Pouteria trees within the cloud forest itself in the current study, however, we have no reliable record of either the time variability or annual climatology of relative humidity.

We use the Barbour et al. (2004) (18) model of the environmental controls on the stable isotope composition of wood, as modified and adapted by Evans (2007) (19), to simulate theoretical monthly stable oxygen isotope climatology. The model has previously been described in detail and deployed for temporal simulations (19, 7). Briefly, the model simulates cellulose $\delta^{18}O$ as a function of temperature, precipitation, and relative humidity data. Many model parameters (18) are only weakly constrained by observations, particularly for tropical species and environments. Uncertainties in these parameters influence the resulting simulation by changing both the mean and standard deviation, and estimates of the $\delta^{18}O$ of cellulose have uncertainties as large as ~1‰ if we assume 20% uncorrelated errors in each of 14 variable parameters (7, 19). Nearly all of this uncertainty arises, however, from a dilution or contraction of the overall time series variance, while year-to-year patterns of variability remain the same (7). We estimated leaf temperature as a function of air temperature (20). Atmospheric water vapor $\delta^{18}O$ is calculated as a function of estimated condensation temperature (21). Stomatal conductance is calculated as a function of the vapor pressure deficit, which is derived from the monthly air temperature, the
derived leaf temperature, and relative humidity. The $\delta^{18}O$ of water at the site of photosynthesis in the leaves is calculated as a change in the source water $\delta^{18}O$ as a function of the kinetic and equilibrium diffusive fractionation, leaf temperature, water vapor $\delta^{18}O$, and the vapor pressure gradient between the leaf and atmosphere.

We use this model to develop hypotheses for the sign and potential magnitude of the response of $\delta^{18}O$ to climate variability. Daily values for temperatures and precipitation from the Campbell station (17) were combined to form monthly mean and total precipitation climatology for model input. For relatively humidity, we extracted a time series of monthly values from the NCEP Reanalysis II gridded data (22, 23) for the latitude, longitude, and atmospheric pressure level (850 mb) which most closely corresponds to the Monteverde Cloud Forest. Following Anchukaitis et al. (2008) (7), we calculated monthly source water $\delta^{18}O$ values as a function of the observed relationship between rainfall amount and the $\delta^{18}O$ composition of rainfall from Rhodes et al. (2006) (11). We regressed the $\delta^{18}O$ on rainfall amounts for those data with aggregate collection periods of two weeks to two months. We determined that shorter collection periods showed a higher variability that might reflect the time of sampling and the trajectory and history of individual weather systems. Longer sampling periods excessively smoothed monthly differences related to the timing of precipitation seasonality. Source water $\delta^{18}O$ values were calculated based on monthly total precipitation values from the Campbell (17) dataset. Our regression model accounts for 56% of the variance in the observed dataset and is significant at $p < 0.01$ with 14 effective degrees of freedom.

We created three hypotheses to predict the response of annual $\delta^{18}O$ cycles to climate variability. First, we calculated an overall mean $\delta^{18}O$ cycle (“control”) by averaging the monthly values from all years with available climate data. We then ran the model with imposed monthly wet season (May—October) precipitation and $\delta^{18}O$ values equal to that of the observed maximum observed rainfall for those months for all years. We also created two mean $\delta^{18}O$ cycles where the dry season relative humidity was replaced by the maximum or minimum observed relative humidity for those months for all years. The mean of the experimental model simulation was set such that the means of the noneexperimental season (dry or wet) was the same as that in the simulation created using the unaltered, observed climate data. In this way the expected interannual magnitude of the model-generated anomalies relative to the control could be isolated for each season (corresponding to the maxima and minima of the annual $\delta^{18}O$ cycle).

These process modeling experiments provide biophysical support for the existence of annual $\delta^{18}O$ cycles in cellulose arising from the annual wet and dry seasons that can be used for chronological control (Fig. S1, compare with observed annual isotope cycle in Fig. 2A). Model experiments demonstrate that anomalies in this cycle of 1‰ or more can be generated by anomalous wet season rainfall or dry season relative humidity observed over the short instrumental or Reanalysis period. Note also that wet season rainfall anomalies carry over to the following dry season and influence the annual cycle amplitude in this manner. Modeling results provide quantitive support for climatic interpretation of isotope variability to complement statistical analysis.

We emphasize that, despite uncertainties in the parameter set for the model (7, 18, 19), these almost entirely result in changes in the mean or standard deviation of the overall time series, but have almost no effect on relative magnitude of the mean $\delta^{18}O$ annual cycle compared to the amplitude anomalies associated with the sensitivity experiments described above. The annual cycle is dominated by the existence of the amount effect in the annual cycle of precipitation $\delta^{18}O$ (19) and the anomalies by changes in relative humidity and precipitation amount. This has been previously documented in other $\delta^{18}O$ cellulose modeling studies at monthly resolution (7, 19). Model code in MATLAB is available via the NOAA NCDC World Data Center, ftp://ftp.ncdc.noaa.gov/pub/data/paleo/treering/northamerica/costarica/evans2007/evans2007.txt. The original Microsoft Excel version of the model (18) is available from the University of Utah, http://ecophys.biology.utah.edu/public/Tree_Ring/.

**Radiocarbon Dating.** Wood and cellulose samples were selected in order to provide high-resolution dates using the $^{14}C$ signature of atmospheric atomic weapons testing (24). Four samples were wood that we preprocessed using an acid-base-acid (ABA) method developed for tropical trees (25, 26). Three samples had been previously processed on cellulose using the Brendel method (27, 28). A replicate sample of ABA treated samples was prepared using the Brendel technique, in order to provide a confirmation of the radiocarbon-bias correction for $\Delta^{14}C$ measurements on cellulose extracted using the Brendel method (28). Extracted wood and cellulose samples were combusted and the purified carbon dioxide reduced to an iron carbide powder over hot zinc for $\Delta^{14}C$ measurement by tandem accelerator mass spectrometer at the University of Arizona AMS Facility (29, 30).

5. Trewdte K et al. (2006) The twentieth century was the wettest period in northern Pakistan over the past millennium. Nature 440:1179–1182.
20. Gonfiantini R, Roche MA, Oliivy JC, Fontes JC, and Zuppi GM (2001) The altitude effect on rainfall amounts for the model rainfall or dry season relative humidity observed over the short instrumental or Reanalysis period. Note also that wet season rainfall anomalies carry over to the following dry season and influence the annual cycle amplitude in this manner. Modeling results provide quantitive support for climatic interpretation of isotope variability to complement statistical analysis.

We emphasize that, despite uncertainties in the parameter set for the model (7, 18, 19), these almost entirely result in changes in the mean or standard deviation of the overall time series, but have almost no effect on relative magnitude of the mean $\delta^{18}O$ annual cycle compared to the amplitude anomalies associated with the sensitivity experiments described above. The annual cycle is dominated by the existence of the amount effect in the annual cycle of precipitation $\delta^{18}O$ (19) and the anomalies by changes in relative humidity and precipitation amount. This has been previously documented in other $\delta^{18}O$ cellulose modeling studies at monthly resolution (7, 19). Model code in MATLAB is available via the NOAA NCDC World Data Center, ftp://ftp.ncdc.noaa.gov/pub/data/paleo/treering/northamerica/costarica/evans2007/evans2007.txt. The original Microsoft Excel version of the model (18) is available from the University of Utah, http://ecophys.biology.utah.edu/public/Tree_Ring/.

Fig. S1. Process model sensitivity experiments. Model simulations demonstrate that anomalies in the annual δ18O cycle are generated by higher than normal wet season rainfall (experiment with an imposed maximum observed wet season rainfall, green line) or dry season relative humidity (experiment with an imposed maximum or minimum observed relative humidity, blue and red lines, respectively). Note that the model predicts that wet season rainfall anomalies carry over to the following dry season and influence the annual cycle amplitude in this manner, whereas anomalies associated with changes in relative humidity are confined to the experimentally induced season itself.

Fig. S2. The sum of dry days during the dry season in consecutive blocks longer than a given threshold, indicated above each plot. Least-squares trends for 1973–2000 are shown by the red lines, and for 1983–2000 by the green lines. The magnitude and significance (p-values are shown for the later period) of the trend is a function of the time period and the chosen threshold.
<table>
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<th>Sample</th>
<th>AA74375</th>
<th>AA74376</th>
<th>AA74377</th>
<th>AA776775</th>
<th>AA74381</th>
<th>AA74379</th>
<th>AA74378</th>
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<tr>
<td>Core</td>
<td>MV12</td>
<td>MV12</td>
<td>MV12</td>
<td>MV15</td>
<td>MV15</td>
<td>MV15</td>
<td>MV15</td>
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<tr>
<td>Depth (mm)</td>
<td>167</td>
<td>230</td>
<td>242</td>
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<td>1.0842</td>
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<tr>
<td>1632 (77.1)</td>
<td>1894</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1905 (15.6)</td>
<td>1909 (16.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1954</td>
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<td>Bayesian calibration</td>
<td>Posteriori 2σ range A.D.</td>
<td>1938–1939</td>
<td>1931–1932</td>
<td>1908–1909</td>
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<tr>
<td>% Agreement</td>
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<td>114.9</td>
<td>67.5</td>
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<tr>
<td>Model agreement (threshold)</td>
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</table>

Radiocarbon analysis from MV12 and MV15. Calibrated dates are shown with the percentage of the probability density function associated with each range of years. Post-bomb calibrated dates include any additional uncertainty associated with using different atmospheric Δ14C curves (31, 32). Bayesian analysis for development of a posteriori probability functions was performed using OxCal, applying the known temporal (stratigraphic) order of the dates and the estimated annual increment based on the δ18O chronology (33, 34). Agreement scores for the Bayesian calibration are uniformly above 60% for the individual dates and exceeds the lower minimum threshold (~35%) for the model as a whole (33). F14C values have been corrected for those samples prepared with the Brendel method (28) and discussed here in the supporting text. Uncertainty due to this correction is substantially smaller than radiocarbon measurement precision in these cases.