

History of Animals using Isotope Records (HAIR): A 6-year dietary history of one family of African elephants

Thure E. Cerling^{a,b,1}, George Wittemyer^{c,d}, James R. Ehleringer^b, Christopher H. Remien^e, and Iain Douglas-Hamilton^{d,f}

Departments of ^aGeology and Geophysics, ^bBiology, and ^cMathematics, University of Utah, Salt Lake City, UT 84112; ^dDepartment of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, CO 80523; ^eSave-The-Elephants, P.O. Box 54667, Nairobi, Kenya; and ^fDepartment of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom

This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected in 2001.

Contributed by Thure E. Cerling, March 3, 2009 (sent for review December 26, 2008)

The dietary and movement history of individual animals can be studied using stable isotope records in animal tissues, providing insight into long-term ecological dynamics and a species niche. We provide a 6-year history of elephant diet by examining tail hair collected from 4 elephants in the same social family unit in northern Kenya. Sequential measurements of carbon, nitrogen, and hydrogen isotope ratios in hair provide a weekly record of diet and water resources. Carbon isotope ratios were well correlated with satellite-based measurements of the normalized difference vegetation index (NDVI) of the region occupied by the elephants as recorded by the global positioning system (GPS) movement record; the absolute amount of C₄ grass consumption is well correlated with the maximum value of NDVI during individual wet seasons. Changes in hydrogen isotope ratios coincided very closely in time with seasonal fluctuations in rainfall and NDVI whereas diet shifts to relatively high proportions of grass lagged seasonal increases in NDVI by ≈2 weeks. The peak probability of conception in the population occurred ≈3 weeks after peak grazing. Spatial and temporal patterns of resource use show that the only period of pure browsing by the focal elephants was located in an over-grazed, communally managed region outside the protected area. The ability to extract time-specific longitudinal records on animal diets, and therefore the ecological history of an organism and its environment, provides an avenue for understanding the impact of climate dynamics and land-use change on animal foraging behavior and habitat relations.

C₄ photosynthesis | carbon-13 | stable isotopes | wildlife conservation

Variation in temporal and spatial resource quality and abundance can have strong effects on animal ecology and community resource partitioning (1). In particular, changes in species composition and abundance at seasonal and longer time scales strongly influences diet and, as a result, community dynamics and the life history of animals (2, 3). Quantifying and dating fine scale foraging behaviors is difficult, typically causing foraging studies to focus on averages compiled from observations and measurements collected from multiple, and often unknown, individuals (e.g., refs. 4–6); such dietary data are difficult to relate to spatially or temporally explicit resource changes. Quantifying the long-term diets of a single individual requires continuous observation, often in the face of cryptic life stages or range shifts and seasonal migration. As such, detailed dietary monitoring through observations is intractable for many species, although the importance of quantifying climate or human mediated diet changes that many species are experiencing is more critical than ever (7). Recent developments in stable isotope ecology enable the derivation of temporally explicit diet records, offering a means by which foraging decisions and the effect of ecological shifts on species can be recorded and compared over time.

Stable isotopes in animal tissues record dietary preferences and ecological conditions experienced by an individual, with substrates such as hair containing longitudinal records of isotope ratios. ¹³C/¹²C, ¹⁵N/¹⁴N, and ³⁴S/³²S ratios record dietary input and habitat characteristics (e.g., refs. 8–11), and D/H and ¹⁸O/¹⁶O ratios record information about water sources (12). One of the most strongly delineated isotopic signals occurs in the ^δ¹³C ratios of plants, using the C₃ and C₄ photosynthetic pathways: Most C₄ plants have ^δ¹³C values between –11 and –14‰, whereas most C₃ plants have ^δ¹³C values between –25 and –29‰. Tropical grasses almost exclusively use the C₄ pathway; most trees, shrubs, and forbs use the C₃ pathway. Therefore, isotope ratios in animal tissues such as hair, which record dietary input, provide a clear indicator of these dietary preferences. Studies of African savanna elephants (*Loxodonta africana*) show that they prefer grass during the wet season but rely on browse during the dry season (6, 13–16). It is anticipated that significant changes in vegetation will take place in sub-Saharan Africa in the next few decades because of resource competition between animals and humans and because of global climate change. Therefore, it is of interest to trace the relationship between elephant diet and vegetation change over a long time interval, particularly in respect to the keystone role elephants play in savanna ecosystems (17, 18).

Here, we present a 6-year chronology of temporally fine-scale data on diet changes of a single elephant family in Samburu-Buffalo Springs National Reserves in northern Kenya. This study builds on our long-term observations of elephants in northern Kenya (19–24), on our interests in understanding isotope incorporation into animal tissues (12, 25–30), and on our interests in applying those principles to wildlife ecology (14, 15, 16). Diet records are interpreted from multiple hair samples collected annually or subannually over the study interval. We compare this diet record to rainfall and remotely sensed records of net primary productivity (NPP) in the form of normalized differential vegetation index (NDVI) data from the same region. Diet shifts are also related to reproductive activity and spatial behavior. With ecological and environmental changes occurring from regional to global scales (including land use change and global climate change) as the result of human activities, shifts in ecological communities are likely to occur. Monitoring diet changes as food resource availability shifts via continuous, long-term isotope records provides an important means to

Author contributions: T.E.C., G.W., J.R.E., and I.D.-H. designed research; T.E.C., G.W., J.R.E., and I.D.-H. performed research; C.H.R. contributed new reagents/analytic tools; T.E.C., G.W., J.R.E., C.H.R., and I.D.-H. analyzed data; and T.E.C. and G.W. wrote the paper.

The authors declare no conflict of interest.

¹To whom correspondence should be addressed. E-mail: thure.cerling@utah.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0902192106/DCSupplemental.

identify and study the effect of environmental change on an animal's ecological interactions.

Results

Background Isotope Values of the Environment: Plants and Water.

Over 250 individual plant samples were collected from October 2004 to July 2005, and in May 2006; this former interval corresponded to a drier than normal period whereas the latter was at the end of a near-normal “long rains” season. The average $\delta^{13}\text{C}$ of C_3 plants was $-27.4 \pm 1.0\text{‰}$ and that for C_4 plants was $-13.4 \pm 1.0\text{‰}$ (Table S1). Ranges in $\delta^{13}\text{C}$ of individual plants and plant species varied by $>3\text{‰}$, generally having more negative values during the wet season and more positive values during the dry season for C_3 plants. No significant correlations between NDVI and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, or ‰N were found for the 10-month collection period.

Nitrogen isotopes showed that only *Indogofera* could be considered to be N-fixing during this period. *Indogofera schimperi* had $\delta^{15}\text{N}$ values near 0‰ ; most of these samples were from the more-xeric upland localities; *I. schimperi* from riparian zones had higher ‰N and higher $\delta^{15}\text{N}$ values ($+3$ to $+9\text{‰}$). All *I. spinosa* samples had high $\delta^{15}\text{N}$ values ($+4$ to $+11\text{‰}$) regardless of ‰N . $\delta^{15}\text{N}$ values of all *Acacia* (*Acacia eliator*, *Acacia reficiens*, *Acacia tortilis*) had elevated $\delta^{15}\text{N}$ values, ranging between $+3.5$ and $+15.9\text{‰}$ and cannot be considered to be exclusively N-fixing plants in this environment. Grasses and sedges generally had high $\delta^{15}\text{N}$ values with average values as high as $+12\text{‰}$ (Table S1).

An 18-month survey of $\delta^{18}\text{O}$ and δD in river water from the Ewaso N'giro showed seasonal fluctuations; δD and $\delta^{18}\text{O}$ values were more negative in the wet season and more positive in the dry season. Deuterium excess [$D_{\text{xs}} = \delta\text{D} - 8 \delta^{18}\text{O}$ (31)] is used to evaluate meteoric waters with respect to the global meteoric water line [GMWL: $\delta\text{D}_{\text{GMWL}} = 8 \delta^{18}\text{O} + 10$; (32)]. Two waters with high D_{xs} values ($>35\text{‰}$) were not included in the trends discussed below. Stable isotope ratios of the Ewaso N'giro during the wet season (NDVI monthly average >0.25) are well correlated ($r^2 = 0.94$) with a trend of $\delta\text{D} = 6.8 \delta^{18}\text{O} + 7.1$, a similar slope and intercept as the GMWL and to other unevaporated meteoric waters in Kenya (33, 34). Waters from the river in the dry seasons (monthly average NDVI < 0.25) show evaporative enrichment of D and ^{18}O : $\delta\text{D} = 4.1 \delta^{18}\text{O} + 3.3$ ($r^2 = 0.81$).

Growth Rates and Correlations Between Members of the Same Family.

Hair was collected and analyzed from 4 different members of the same family unit; Royals: mother Queen Elizabeth (M1) and siblings Victoria (M2), Cleopatra (M4), Anastasia (M5), and growth rates were calculated using the method of Wittemyer (16). Growth rates of the tail hairs were between 0.73 and 1.04 mm/day. We found little evidence for variable growth rates within a single hair even during periods of high metabolic stress related to reproduction (16). Individual hair samples were up to 580 mm long (median: 460 mm) and had individual chronologies covering up to 20 months (median: 18 months). The correlation between isotope chronologies between different familial members was very high ($r^2 = 0.79$), slightly lower than correlations between hairs collected from a single individual ($r^2 = 0.89$ to 0.93) but higher than two individuals from different family units ($r^2 = 0.44$; ref. 16). Fig. S1 shows the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ records for hair samples collected from M4 and M5 in July 2004.

Isotope ratios from dung samples collected on the same days from M4 and M5 were also highly correlated [reduced major axis (RMA): $\delta^{13}\text{C}(\text{M5}) = 1.133 \delta^{13}\text{C}(\text{M4}) - 4.79$; $r^2 = 0.68$; $n = 13$]. Unfortunately, few samples were collected during the wet season, when the greatest variance in isotope values occurred, because the focal elephants dispersed to regions inaccessible to our field team during these periods.

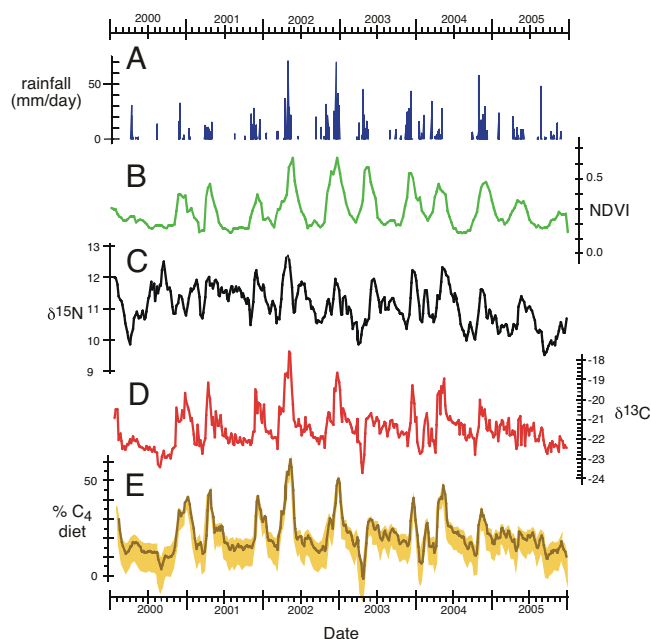


Fig. 1. Nitrogen and carbon isotope data and the estimated percentage of C_4 biomass in diet derived from the composite hair profile demonstrate seasonal oscillations matching fluctuations in rainfall and NDVI from the Samburu region. (A) Rainfall from Archer's Post. (B) NDVI of Samburu region. (C) $\delta^{15}\text{N}$ of composite hair profile. (D) $\delta^{13}\text{C}$ of composite hair profile. (E) Estimated percentage of C_4 biomass in the diet (3-point running average). The solid line is using the model as described in *Methods*, and the C_3 and C_4 end-member values of -27.4 and -13.4‰ , respectively. Shaded envelope gives ranges encompassed by seasonal end-member C_3 and C_4 values of -28.5‰ and -12‰ (wet season top of envelope), respectively, and -26‰ and -14‰ (dry season bottom of envelope), respectively.

Diet History of Royals Family Unit Based on Carbon Isotopes.

Because of the high correlation and overlap between different hairs collected from different individuals within the same family unit, a long-term composite diet history of the “Royals” family unit was compiled using the isotope profiles from hairs of different individuals. Fig. 1 shows rainfall, NDVI, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and estimated C_4 biomass in diet for the 6-year period from 2000 through the beginning of 2006. Biomass estimates were calculated using 3 different C_3 and C_4 mixing model end-members. The nominal case, shown as a solid line in Fig. 1E, uses the average $\delta^{13}\text{C}$ values for the >250 plant samples from the Samburu. C_3 plants tend to become more positive and C_4 plants tend to be slightly more negative in xeric environments (35, 36); thus the mixing lines are likely to be slightly compressed during the dry season and slightly expanded in the wet season compared with the nominal case. To better assess the possible variability in actual C_4 biomass contributions, we used C_3 and C_4 end-member values based on samples collected during the wet and dry seasons of -28.5‰ and -12‰ (“wet-season” mixing line) and -26 and -14‰ (“dry-season” mixing line). This analysis has an important consequence: baseline (i.e., the lower “floor” of C_4 biomass intake) values of C_4 biomass for the nominal and “wet-season” cases are ≈ 15 – 20% C_4 biomass, whereas for the “dry-season” case the baseline is $\approx 5\%$ C_4 biomass (Fig. 1E). However, the maximum C_4 intake during the wet season is little affected by these different mixing line values because of the “cross-over” of the mixing lines occurs at $\approx 50\%$ C_4 biomass (i.e., the 50% C_4 values for the wet season case, nominal case, and dry season case are -20.5‰ , -20.4‰ , and -20‰ , respectively). Average percentage of C_4 biomass intake over the entire 6-year period is estimated to be 24%, 21%, and 12% for the “wet season,” “nominal,”

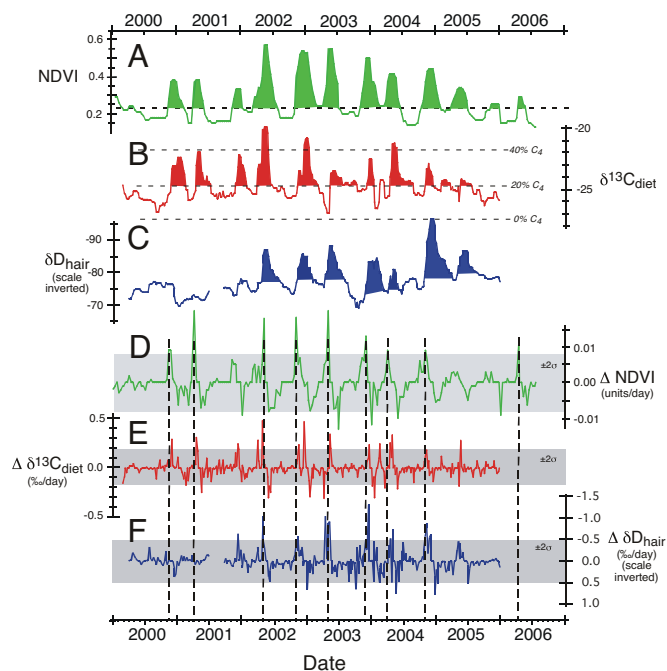


Fig. 2. Median values for NDVI, $\delta^{13}\text{C}_{\text{diet}}$, and $\delta\text{D}_{\text{hair}}$. All values are ≈ 1 month medians. (A) Median values of NDVI. (B) Median values of $\delta^{13}\text{C}$ in diet. (C) Median values of δD in hair (scale inverted). (D) First derivative of NDVI record: ΔNDVI (units are NDVI/day). (E) First derivative of $\delta^{13}\text{C}$ record: $\Delta\delta^{13}\text{C}$ (units are $\text{‰}/\text{day}$). (F) First derivative of δD of hair: $\Delta\delta\text{D}$ (units are $\text{‰}/\text{day}$). (D–F) Shaded horizontal boxes show $\pm 2\sigma$ for ΔNDVI , $\Delta\delta^{13}\text{C}$, and $\Delta\delta\text{D}$; dashed vertical lines show the maximum values ($> 2\sigma$) for ΔNDVI .

and “dry season” cases, respectively, with maximum intake values between 58% and 60% for all 3 cases occurring in May 2002.

Results of Nitrogen Isotope Analyses. $\delta^{15}\text{N}$ values for hair from the Royals family unit are shown in Fig. 1C. $\delta^{15}\text{N}$ tends to have high values when $\delta^{13}\text{C}$ values are also high (with correlation coefficients of $r^2 > 0.7$ for periods of 3 months or longer), demonstrating that C_4 plants tend to have a higher $\delta^{15}\text{N}$ value in the ecosystem as corroborated from vegetation samples presented previously. This is true for the single-day survey of plants and for dung samples: $\delta^{15}\text{N}$ in dung is positively correlated with $\delta^{13}\text{C}$ [RMA: $\delta^{15}\text{N}_{\text{dung}} = 0.39 \delta^{13}\text{C}_{\text{dung}} + 16.2$ ($r^2 = 0.63$; $n = 26$)]. Sponheimer (25) observed 0.8‰ depletion in $\delta^{13}\text{C}$ in dung compared with diet for several different domestic species (cattle (*Bos taurus*), goat (*Capra hircus*), alpaca (*Lama pacos*), llama (*Lama glama*), rabbit (*Oryctolagus cuniculus*)). $\delta^{15}\text{N}$, however, is enriched in ^{15}N compared with the diet by 0.5 to 3‰ (26, 37, 38). Elephant dung consist of poorly digested material so that the isotope fractionation during digestion is likely to be less than in animals with a more efficient digestive physiology.

Hydrogen and Oxygen Isotope Results from Hair. Fig. 2 shows the record of NDVI, $\delta^{13}\text{C}_{\text{diet}}$ and $\delta\text{D}_{\text{hair}}$ for the ≈ 6 -year period presented as the median value over 1-month periods; here we also provide the first derivatives of each of these records (ΔNDVI , $\Delta\delta^{13}\text{C}_{\text{diet}}$, $\Delta\delta\text{D}_{\text{hair}}$, respectively). There are 9 periods where the ΔNDVI exceeds the 2σ (standard deviation) value, and 1 where it approaches the 2σ value (Fig. 2D). These are the periods of most rapid change in the vegetation index and serve as a reference for changes in carbon and hydrogen isotope changes in hair.

Discussion

Long-Term Diet, NDVI, and Rainfall. The fraction of dietary C_4 biomass in the Royals family unit was strongly seasonal, with

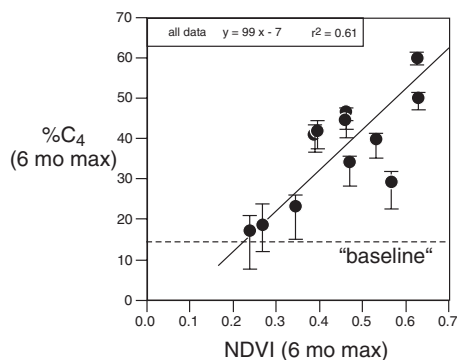


Fig. 3. Correlation between maximum NDVI and maximum percentage of C_4 biomass calculated in diet for 6-month time intervals from 2001 to 2006; reduced major axis (RMA) used to calculate regression. Time intervals were from 1 March to 1 September and from 1 September to 1 March, so as to differentiate the “long” and “short” rains, respectively (usually April–May and November–December). The solid line is for all data and vertical bars show the total range of calculated diets, using the 3 end-member mixing values discussed in *Diet history of Royals Family*.

rainy season $\delta^{13}\text{C}_{\text{diet}}$ values generally 3‰ to 6‰ higher than in the dry season. This corresponds to a baseline diet between 5% and 20% C_4 biomass (Fig. 1E) in the dry season to peaks between 40% and 60% C_4 biomass in the wet season for this family unit. To compare maximum NDVI and maximum percentage C_4 contribution to the elephant diets over the 6-year interval we considered the periods from 1 March to 1 September and 1 September to 1 March to capture the 2 rainy seasons in this environment. The fraction of C_4 biomass in the diet (i.e., C_4 -grasses) is highly correlated with NDVI (Fig. 3; RMA: $\% \text{C}_4 = 99 * \text{NDVI} - 7$; $r^2 = 0.61$). Therefore, the fraction of C_4 biomass in the diets of savanna elephants is highly dependent on seasonal rain and net primary productivity (NPP). During periods of low NPP, the maximum percentage of C_4 biomass was $< 30\%$, whereas during favorable periods it exceeded 40% for this family unit. The minimal increase in dietary grass during the droughts of 2000 and in late 2005, as exhibited by the NDVI and the stable isotope records, provides further evidence of the strong relationship between NPP/rainfall and diet among elephants.

The timing of diet in forage and water isotopic shifts are strongly related to changing ecological conditions, as exemplified by the synchronicity of the maximum rates of increase, peaks, and maximum rates of decrease among rainfall, NDVI, $\delta\text{D}_{\text{hair}}$, and $\delta^{13}\text{C}_{\text{diet}}$ during the 6-year study (Fig. 4). These temporal relationships are compared with the timing of reproduction in the greater Samburu population (22, 29). Seasonal diet changes were abrupt at the beginning of the wet season but tapered off gradually at the end of the wet season (Fig. 4D). Changes in $\delta^{13}\text{C}_{\text{diet}}$ track the chronology of NDVI values but lags changes in NDVI by several weeks. The temporal lag between $\delta^{13}\text{C}_{\text{diet}}$ and NDVI was the same when comparing either NDVI_{max} with $\delta^{13}\text{C}_{\text{max}}$ or $\Delta\text{NDVI}_{\text{max}}$ with $\Delta\delta^{13}\text{C}_{\text{max}}$ (Figs. 2 and 4). Changes in δD are more closely synchronized with NDVI, where the maximum rate of increase and peak occur simultaneously (Fig. 4). The strong synchronicity between NDVI and $\delta\text{D}_{\text{hair}}$, which lags the onset of seasonal rains, demonstrates the importance of changing water sources associated with the seasonal rains; this could be in the form of new or replenished drinking water supplies and forage-derived water. The delay between changes in NDVI and diet switching appears in part to relate to the handling time associated with short, early season grasses; the new-growth grass must grow to a certain height before it can readily be grasped by the trunk (G.W., personal observation). In

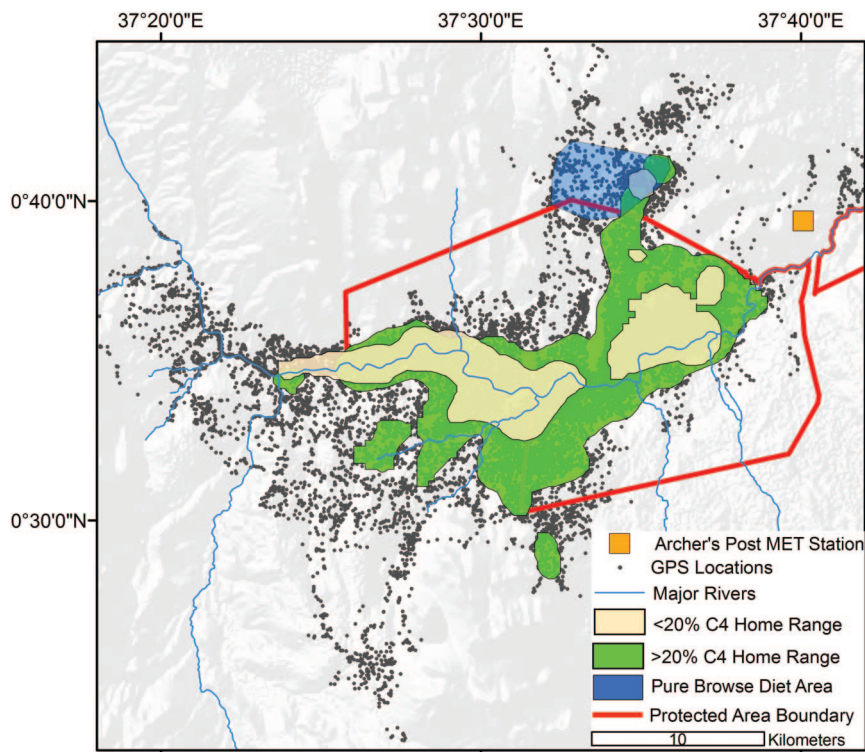


Fig. 6. Regional map displaying GPS positions (black dots) collected from M4 and M5 in and around the national reserves (red polygon overlaid on a digital elevation map) during the 6-year study. The 95% kernel home range (42) retracted to areas in close proximity of the permanent Ewaso N'giro river (blue line) during dry season periods when diets were <20% grass (beige polygon), whereas ranges expanded to regions further from the river as the proportion of grass increased (green polygon). The only period when diets were 100% browse (blue polygon) occurred north of the protected area boundary in a heavily grazed community conservation region.

observed in the vegetation survey was *I. schimperi*, a favorite C_3 food item of elephants in every season.

Spatial Distribution, NDVI, and Diet Change. The movements of at least 1 member of the Royals family unit were recorded by a global positioning system (GPS) collar the entire length of this study. Fig. 6 shows the 95% kernel (42) incorporating the location of the Royals family unit. During periods of low (<20%) C_4 intake the family unit was usually within ≈ 2 km of the riparian corridor of the Ewaso N'giro (Fig. 6). During periods when C_4 biomass was a high portion of the diet (>20% C_4), the family unit was found much further from the riparian corridor, and was more commonly found outside the Samburu and Buffalo Springs National Reserve boundaries (Fig. 6). These linkages and covariances between water and food distributions have significant management implications, particularly when interannual shifts in seasonal precipitation alter availability of C_4 food resources.

Cattle are generally excluded from the Samburu and Buffalo Springs Reserves, resulting in little competition between elephants and livestock for resources within these protected areas. Yet the stable isotope data suggest periods when these animals may compete for resources. Late April to early May 2003 was a time of anomalous diet for the Royals family unit (Figs. 1 and 2, and Fig. S1). During this apparently normal rainy season, the Royals family unit did not switch to grazing as was normal, but had a diet that was virtually 100% C_3 ; in fact, it was the most negative $\delta^{13}C$ value for the entire 6-year observation period (Figs. 1 and 2). During this period GPS data showed that the elephants were mostly beyond the northern edge of Samburu Reserve, in an area heavily used by the local pastoralists (Fig. 6). The region is an *Acacia mellifera* and *A. reficiens* woodland subject to heavy overgrazing by livestock. Although the reasons

for use of this area by the Royals family unit are not known, the impact of overgrazing by cattle on the typical wet season diet of elephants is clear; competition with cattle results in poor access to high quality grass forage because cattle keep the grass very short and out-compete elephants for this resource. This example shows that human activities have a large impact on elephant diets and ecological function.

Conservation Implications. The findings presented here have important implications for management of elephant populations in relation to global climate change on one scale, and local land use change at another scale. NDVI can change over the long term for many reasons, but increasing temperature in regions with minimal rainfall is likely to decrease NPP and NDVI. Similarly, changes in land use due to increased stocking rates as human population increases can result in changes in NDVI.

Elephants are a keystone species in savanna ecosystems, shaping the relative densities of grass and woody vegetation (17, 40). In many ecosystems, elephant range restriction concentrates and amplifies impacts of elephants on vegetative communities, leading to effects on species composition across trophic levels (43, 44). With climate models predicting greater variation in annual rainfall in Eastern Africa (45), the dynamics between elephant populations and their environments are likely to be strongly regulated by the relationship between climatic fluctuation and diet. During droughts, the combination of constrictions of feeding ranges to areas with permanent water (Fig. 6) and increased reliance on woody vegetation by elephants is likely to extenuate the impacts on vegetative species age distributions and composition (46). In addition, elephant ranging behavior, sociality and reproduction are strongly mediated by climate variation (22–24). Climatic-induced diet changes may alter re-

cruitment regimes—driving more pronounced seasonality in conceptions related to the proportion of C_4 vegetation in female diets.

Conclusions

Long-term diet histories of mammals are recorded in animal tissues such as hair, and these histories can be interpreted in the context of stable isotope ecology. A 6-year history of a single elephant family unit in northern Kenya shows that seasonal diet changes are well correlated with changes in NDVI. During the dry season, $\delta^{13}C$ values have a baseline value that indicates a diet composed of 5% to 20% C_4 biomass depending on the values used for end-member C_3 and C_4 vegetation. The absolute amount of grass consumption in any wet season is well correlated with the maximum value of NDVI associated with that wet season, and the peak in grass consumption occurs ≈ 2 weeks after the peak in NDVI. These results show the adaptability of elephants in the face of climate change. However, long-term changes in NDVI, whether due to land use change, competition with livestock, or to long-term climate trends, are likely to be accompanied by changes in the amount of grass available to be consumed by elephants during the wet season—shaping the ecological role played by elephants across their range.

Changes in the δD of hair are well correlated with the isotope composition of local drinking water, and the minimum value for δD occurs at essentially the same time as the peak NDVI. The high temporal resolution of hair δD can be used as a unique ecological tracer in future research. Not only can δD accurately identify the timing of seasonal resource changes, but coupling this data with fine-scaled spatial information on water sources can offer new directions of research regarding animal behavior.

Tail hair of wild animals represents an archive of dietary behavior that provides an opportunity to quantify diet and the environmental conditions experienced by those animals. This archive can be accurately dated and is closely linked to local climate parameters. Such information can be used to reconstruct historic climatic events at fine temporal scales and spatial scales if coupled with GPS observations. It also provides insights to resource use and environmental interaction not previously accessible for animal ecological and evolutionary studies.

Methods

Study Area and Population. The elephants (*L. africana*) sampled in this study inhabit the region in and around the 220 km² Samburu and Buffalo Springs National Reserve in northern Kenya (37.5° E 0.5° N). These semiarid parks are dominated by *Acacia-Commiphora* savanna and scrub bush and located along the Ewaso N'giro River (Fig. 7), the major permanent water source in the region (47). Rainfall averages ≈ 350 mm per year and occurs during biannual wet seasons that generally take place in April/May and November/December; Archer's Post (Fig. 6) is the nearest meteorological station. The elephants using these reserves (Fig. 7) are individually identified, following well-established methods (19), allowing hair sampling from the same individual across time. For a more detailed description of the study population and ecology of the study area, see ref. 16.

GPS radio collars were fitted to elephants in the Samburu National Reserve, northern Kenya, between 2001 and 2006 (20). Collars were programmed to record positions at hourly intervals, offering detailed records of movement. Tail hairs (Fig. 7) from each elephant were collected during immobilization operations while the collars were being fitted, and later when batteries were being changed or when the collars were being removed. We sampled 4 different breeding females from the same family unit (Royals), known to maintain direct proximity with each other $>80\%$ of the time (21). Two of the sampled females, M4 (Anastasia) and M5 (Cleopatra), were fitted simultaneously with GPS radio collars for a 6 month period, during which they spent $>95\%$ of the time within 1 km of each other (80% within 250 m), used identical ranges, and moved similar daily distances. Three of the family unit members, including the 2 radio-tracked females, were siblings, daughters of the 4th (M1: Queen Elizabeth), who died in 2000.

Dung samples were collected during observational transects during the years 2004–2005. Plant samples were collected from 1 riparian zone 3 times

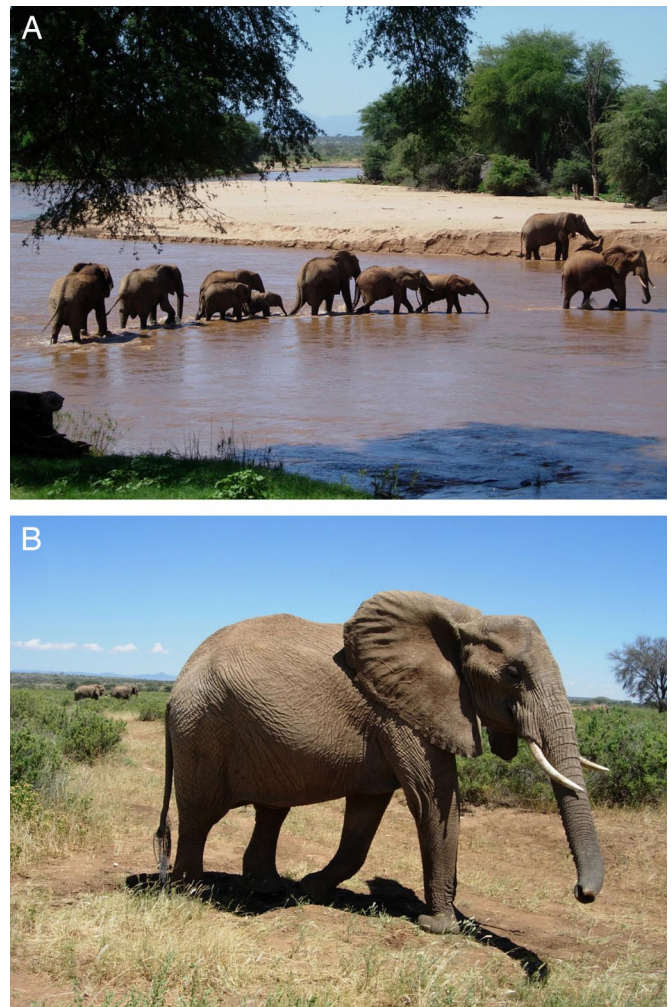


Fig. 7. (A) Elephant family crossing the Ewaso N'giro in Samburu and Buffalo Springs Reserve (photo by M. Kephart). (B) Elephant with tail hair typical of that used in this study.

per month, for 10 months from October 2004 through July 2005. A single collection of plants was made on a single day during the wet season (21 May 2006); we visited 14 sites ranging from riparian zones to upland bush zones. Water samples were collected for 18 months, 1 sample monthly, from the Ewaso N'giro River from October 2001 to March 2003.

Laboratory Methods. Hair samples were wiped with acetone to remove dirt, grit, and oils. Hair samples were serially sampled, with 1 sample collected from each 5-mm interval for $\delta^{13}C$ and $\delta^{15}N$ analysis (≈ 500 μ gr); the same segments were used for δD and $\delta^{18}O$ organic analysis (≈ 150 μ gr). Five mm corresponds to ≈ 6 days for these samples. Dung and plant samples were oven-dried at 80 °C for 24 h, homogenized, and ground before analysis.

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of elephant hair, dung, and plant material were measured on an isotope ratio mass spectrometer (IRMS: Finnegan 252) after combustion in a flow-through modified Carlo-Erba system. Values are reported using the conventional permil (‰) notation where:

$$\delta^{13}C = \left(\left(\frac{^{13}C/^{12}C}{^{13}C/^{12}C}_{\text{standard}} \right) - 1 \right) \times 1,000,$$

an analogous terminology describes D/H, $^{15}N/^{14}N$, and $^{18}O/^{16}O$ ratios. Standards are V-PDB and AIR for $\delta^{13}C$ and $\delta^{15}N$, respectively, and V-SMOW for δD and $\delta^{18}O$. Isotope enrichment between hair and diet is $\approx 3\%$ for both $\delta^{13}C$ and $\delta^{15}N$. For δD and $\delta^{18}O$ analysis of hair, ≈ 150 μ g of hair from samples or internal standards were equilibrated with water vapor in the laboratory atmosphere, desiccated under vacuum for 7 days, and analyzed. Internal standards were used to correct for exchangeable H (48). δD and $\delta^{18}O$ were analyzed by pyrolyzation of the hair to H_2 and CO in an elemental analyzer furnace (TC-EA) and analyzed for 2H and ^{18}O content, using an IRMS operating in continuous

mode (49). Hair samples were analyzed using a zero-blank carousel to prevent isotope exchange during the analysis period. Results are presented in the ‰ notation, using V-SMOW as the standard. Water analyses were analyzed using Zn-reduction to produce H₂ gas and by CO₂ equilibration for δ¹⁸O before analysis using IRMS.

Data Analysis. We used 10-day composite NDVI data available through Satellite Probatoire d'Observation de la Terre (SPOT) to determine NPP changes in season across the study area. NDVI is a remote sensing index value calculated as the ratio between red and near infrared reflection that is highly correlated with green (photosynthetically active) biomass (50, 51). Remotely sensed data provides a direct measure of photosynthetic activity over large spatial regions, offering advantages over the classically-used point sampled rainfall data in areas, like the study region, where weather stations are sparse (39). Isotope profiles for each elephant were compared with longitudinal 10-day NDVI records to determine the impact of seasonality on diet.

Growth rates of hairs were determined by comparing overlapping stable isotope patterns of δ¹³C and δ¹⁵N (14, 16) and are independent of NDVI measurements, rainfall, and observational data (e.g., births, pregnancies). We estimated the dietary components of C₃ and C₄ biomass, using the model of Cerling et al. (29); we assume the same parameters for isotope turnover pools as determined by Ayliffe et al. (28) because these parameters are not yet known for elephants and previous analysis demonstrated only minor variation in model parameters provided plausible diet estimates (16). In the dietary analysis, we used portions from 8 different hairs to make a single composite reference for the Royals family unit. For C₃ and C₄ end-member δ¹³C values, we use the values of −27.4 and −13.4‰, respectively (unless otherwise specified), as determined from average δ¹³C values of >250 plants sampled in the local ecosystem during the course of this study.

We estimated the δ¹⁵N diet using the isotope turnover model of Ayliffe et al. (28), using the same turnover parameters for N as for C; we use a 3‰ enrichment for diet-hair (27). The parameters are not known but we assume

that similar skeletal elements of amino acids are correlated for amino acids involved in hair growth.

Seasonal peaks in NDVI, δD, and δ¹³C of diet (related to C₄ biomass) were compared across seasons by estimating the major elements of the shape of their respective seasonal cycles: distinguishing initiation (or wet season onset), maximum increase, peak value, maximum decrease, and end point (or dry season onset), using a 5-point median plot (median value, ≈1 month), which preserves peaks in rates of change. NDVI and δ¹³C increase during the wet season, whereas δD of drinking water decreases during these intervals and is therefore plotted using an inverted scale. The peak values for NDVI, δD, and δ¹³C diet were tabulated; the periods of maximum change was determined from the first derivative of the peak curve. For peak initiation we used a threshold value of 0.23 for NDVI, 20% C₄ diet component for δ¹³C_{diet}, and <4‰ below the previous peak value for δD. Peak ends were considered to be the threshold value of 0.23 for NDVI, 20% C₄ diet component for δ¹³C_{diet}, and a return to the peak initiation value for δD. Regressions between stable isotopes are calculated using the reduced major axis (RMA) because relative errors in the dependent and independent variables are of similar magnitude (52, 53). No distinction was made between peaks associated with the “long” versus “short” rains [during the period 2000–2005 the “long rains” and “short rains” had average rainfall values of 143 mm (*n* = 6; max: 259 mm; min: 63 mm) and 193 (*n* = 6; maximum 356 mm; minimum: 55 mm), respectively].

ACKNOWLEDGMENTS. We thank David Daballen, Daniel Lentipo, and Chris Leadismo for assisting in the collection of samples and in observations in the field; Leslie Chesson, Brad Erkkila, Scott Hynek, Todd Robinson, and Jared Singer for assistance in laboratory; Mahala Kephart for photography; the Government of Kenya for permission to carry out this research; and Daryl Codron and Antoine Zazzo for their critical reading of the manuscript. Field work was supported by the Save-The-Elephants and the Packard Foundation. Isotope measurements were done in the Stable Isotope Ratio Facility for Environmental Research laboratory at the University of Utah. This work was carried out under Convention on International Trade In Endangered Species permits US831854, 02US053837/9, and 08US159997/9.

1. Stearns SC (1992) *The Evolution of Life Histories* (Oxford Univ Press, Oxford).
2. Ostfeld RS, Keasing F (2000) Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends Ecol Evol* 15:232–237.
3. Yang LH, Bastow JL, Spence KO, Wright AN (2008) What can we learn from resource pulses? *Ecology* 86:621–634.
4. Talbot LM (1962) Food preferences of some East African wild ungulates. *East African Agri Forest J* 27:131–138.
5. Field CR (1972) The food habits of wild ungulates in Uganada by analysis of stomach contents. *East African Wildlife J* 10:17–42.
6. Codron J, et al. (2006) Elephant (*Loxodonta africana*) diets in Kruger National Park, South Africa: Spatial and landscape differences. *J Mammal* 87:27–34.
7. Nicole S, Worby A, Leaper R (2008) Changes in the Arctic sea ice ecosystem: Potential impacts on krill and baleen whales. *Marine Freshw Res* 59:361–382.
8. DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506.
9. DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 45:341–351.
10. O'Connell TC, Hedges REM (1999) Investigations into the effect of diet on modern human hair isotopic values. *Amer J Phys Anthro* 108:409–425.
11. Macko SA, et al. (1999) Documenting the diet in ancient human populations through stable isotope analysis of hair. *Phil Trans R Soc Ser B* 354:65–76.
12. Ehleringer JR, et al. (2008) Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proc Natl Acad Sci USA* 105:2788–2793.
13. Kingdon J (1979) *An Atlas of Evolution in Africa. Vol III B Large Mammals* (Univ of Chicago Press, Chicago).
14. Cerling TE, et al. (2004) Orphans' tales: Seasonally dietary changes in elephants from Tsavo National Park, Kenya. *Palaeogeogr Palaeoclim Palaeoecol* 206:367–376.
15. Cerling TE, et al. (2006) Stable isotopes in elephant hair documents migration patterns and diet changes. *Proc Natl Acad Sci USA* 103:371–373.
16. Wittemyer G, Cerling TE, Douglas-Hamilton I (2009) Establishing chronologies from isotopic profiles in serially collected animal tissues: An example using tail hairs from African elephants. *Chem Geol*, 10.1016/j.chemgeo.2008.08.010.
17. Laws RM (1970) Elephants as agents of habitat and landscape change in East Africa. *Oikos* 21:1–15.
18. Owen-Smith N (1992) *Megaherbivores: The influence of very large body size on ecology* (Cambridge Univ Press, Cambridge, UK).
19. Wittemyer G (2001) The elephant population of Samburu and Buffalo Springs National Reserves, Kenya. *African J Ecol* 39:357–365.
20. Douglas-Hamilton I, Krink T, Vollrath F (2005) Movements and corridors of African elephants in relation to protected areas. *Naturwissenschaften* 92:163–168.
21. Wittemyer G, Douglas-Hamilton I, Getz WM (2005) The socio-ecology of elephants: Analysis of the processes creating multi-tiered social structures. *Anim Behav* 69:1357–1371.
22. Wittemyer G, Rasmussen HB, Douglas-Hamilton I (2007a) Timing of conceptions and parturitions in relation to NDVI variability in free-ranging African elephant. *Ecography* 30:42–50.
23. Wittemyer G, Ganswindt A, Hodges K (2007b) The impact of ecological variability on the reproductive endocrinology of wild female African elephants. *Horm Behav* 51:346–354.
24. Wittemyer G, Getz WM, Vollrath F, Douglas-Hamilton I (2007c) Social dominance, seasonal movements, and spatial segregation in African elephants: A contribution to conservation behavior. *Behav Ecol Sociobiol* 61:1919–1931.
25. Sponheimer M, et al. (2003a) An experimental study of carbon isotopes in the diets, feces and hair of mammalian herbivores. *Can J Zool* 81:871–876.
26. Sponheimer M, et al. (2003b) An experimental study of nitrogen flux in llamas: Is ¹⁴N preferentially excreted? *J Archaeol Sci* 30:1649–1655.
27. Sponheimer M, et al. (2003c) Nitrogen isotopes in mammalian herbivores: Hair δ¹⁵N values from a controlled-feeding study. *Intern J Osteol* 13:80–87.
28. Ayliffe LK, et al. (2004) Turnover of carbon isotopes in tail hair and breath CO₂ of horses fed an isotopically varied diet. *Oecologia* 139:11–22.
29. Cerling TE, et al. (2007) Determining biological tissue turnover using stable isotopes: The reaction progress variable. *Oecologia* 151:175–189.
30. Podlesak DW, et al. (2008) Turnover of oxygen and hydrogen isotopes in the body water, CO₂, hair and enamel of a small mammal after a change in drinking water. *Geochim Cosmochim Acta* 72:19–35.
31. Gat JR (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. *Ann Rev Earth Planet Sci* 24:225–262.
32. Craig H (1961) Isotopic variations in meteoric waters. *Science* 133:1702–1703.
33. Barton CE, Solomon DK, Bowman JR, Cerling TE, Sayer MD (1987) Chloride budgets in transient lakes: Lakes Baringo, Naivasha, and Turkana, Kenya. *Limnol Oceanogr* 32:745–751.
34. Gonfiantini R, Roche M-A, Olivry J-C, Fontes J-C, Zuppi GM (2001) The isotopic effect on the altitudinal composition of tropical rains. *Chem Geol* 181:147–167.
35. Ehleringer JR, Cooper TA (1988) Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia* 76:562–566.
36. Buchmann N, Brooks JR, Rapp KD, Ehleringer JR (1996) Carbon isotope composition of C₄ grasses is influenced by light and water supply. *Plant Cell Environ* 9:392–402.
37. Steele KW, Daniel RMJ (1978) Fractionation of nitrogen isotopes by animals: A further complication to the use of variations in the natural abundance of ¹⁵N for tracer studies. *J Agri Sci* 90:7–9.
38. Sutoh M, Koyama T, Yoneyama T (1987) Variations in natural ¹⁵N abundances in the tissues and digesta of domestic animals. *Radioisotopes* 36:74–77.
39. Rasmussen HB, Wittemyer G, Douglas-Hamilton I (2006) Predicting time-specific changes in demographic processes using remote-sensing data. *J Appl Ecol* 43:366–376.
40. Dublin HT, Sinclair ARE, McGlade J (1990) Elephants and fire as causes of multiple stable states in the Serengeti Mara Woodlands. *J Animal Ecol* 59:1147–1164.
41. Bowen GJ, et al. (2009) Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. *Amer J Phys Anthro*, in press.

42. Worton BJ (1989) Kernel methods for estimating the utilization distribution in home range studies. *Ecology* 70:164–168.
43. Ogada DL, Gadd ME, Ostfeld RS, Young TP, Keesing F (2008) Impacts of large herbivorous mammals on bird diversity and abundance in an African savanna. *Oecologia* 156:387–397.
44. Guldemond R, Van Aarde R (2008) A meta-analysis of the impact of African elephants on savanna vegetation. *J Wildlife Management* 72:892–899.
45. IPCC (2007) In *Regional Climate Projections*, eds Solomon S, et al. (Cambridge Univ Press, Cambridge, UK), Ch 11.
46. Western D (2007) A half a century of habitat change in Amboseli National Park, Kenya. *African J Ecol* 45:302–310.
47. Barkham JP, Rainy ME (1976) Vegetation of Samburu-Isiolo game reserve. *East Afr Wildlife J* 14:297–329.
48. Bowen GJ, Chesson L, Nielson K, Cerling TE, Ehleringer JR (2005) Treatment methods for the determination of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of hair keratin by continuous-flow isotope ratio mass spectrometry. *Rapid Commun Mass Spectro* 19:2371–2378.
49. Gehre M, Geilmann H, Richter J, Werner RA, Brand WA (2004) Continuous flow $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ analysis of water samples with dual inlet precision. *Rapid Comm Mass Spectrom* 18:2650–2660.
50. Diallo O, Diouf A, Hanan NP, Ndiaye A, Prevost Y (1991) AVHRR monitoring of savanna primary production in Senegal, West Africa. 1987–1988. *Int J Remote Sensing* 12:1259–1279.
51. Goward SN, Prince SD (1995) Transient effects of climate on vegetation dynamics: Satellite observations. *J Biogeogr* 22:549–564.
52. Borradaile GJ (2003) *Statistics of Earth Science Data: Their Distribution in Time, Space and Orientation* (Springer, Berlin).
53. Trauth MH (2007) *MATLAB Recipes for Earth Sciences* (Springer, Berlin).