Stable isotope and DNA evidence for ritual sequences in Inca child sacrifice

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Four recently discovered frozen child mummies from two of the highest peaks in the south central Andes now yield tantalizing evidence of the preparatory stages leading to Inca ritual killing as represented by the unique *capacocha* rite. Our interdisciplinary study examined hair from the mummies to obtain detailed genetic and diachronic isotopic information. This approach has allowed us to reconstruct aspects of individual identity and diet, make inferences concerning social background, and gain insight on the hitherto unknown processes by which victims were selected, elevated in social status, prepared for a high-altitude pilgrimage, and killed. Such direct information amplifies, yet also partly contrasts with, Spanish historical accounts.

ancient DNA | bioarchaeology | South America | stable light isotopes | ice mummies

In 1996, the frozen remains of a 15-year-old girl ("Sarita") were found at the 5,500-m summit of Volcán Sara Sara in Peru (latitude, 14° 48′ S; longitude, 73° 36′ W) (1, 2). In 1999, an Inca shrine 25 m from the 6,739-m summit of Volcán Llullaillaco in northwest Argentina (latitude, 24° 43′ 17″ S; longitude, 68° 32′ 15″ W; currently the world's highest archaeological site[l]) revealed the bodies of another 15-year-old girl (the "Llullaillaco Maiden"[m]) along with a 7-year-old boy ("Llullaillaco Boy") and a 6-year-old girl ("Lightning Girl") (1, 3–5). The shrines from which they come belong to a group of well over 100 Inca ritual sites recorded between 5,200 m and 6,700 m on significant peaks throughout the Andean mountain range, some of which have also yielded preserved remains of ritually killed *capacocha* children; these mountains include Aconcagua (6), Ampato (1), Chuscha (7), and El Plomo (8, 9).

Using scalp hair from the victims' heads and hair found in small accompanying bags, we have examined both synchronic and diachronic data (≈2.5 years worth of detailed information, as in the longest fibers derived from the Llullaillaco Maiden) to answer fundamental questions concerning the children's social and genetic background: how they were selected, the ritual schedule involved, the spatial dimensions of their final pilgrimages, and their proximal causes of death. These data provide a unique opportunity to investigate imperial Inca ceremonies from the perspective of the victims through the use of high-resolution[n] stable light isotope and DNA techniques and to assess the degree of congruence with the Spanish historical accounts that, after Pizarro's conquest of the Inca capital, Cuzco, in A.D. 1533, have provided the primary framework for our understanding of Inca religious practices. The data offer direct insight into the overall mechanism of Inca ritual killing in extreme environments and its significance as a mechanism for social control.

The high peaks of the Andes were sacred to the Inca, associated or even identified with their major deities, such as the weather god, Illapa. Some of the chosen locations are both high enough to attract regular lightning strikes (Lightning Girl is so named because her corpse has sustained a direct hit) and some are active volcanoes, from which smoke, noise, and fire could be at times observed, phenomena easily interpretable within superstitious or religious frameworks.[o] Human sacrifice at significant peaks reinforced reverence for locally sacred mountains (10) and helped legitimize the expanding empire (4) as its population rose to ≈10 million (11). The construction of the shrines and the conducting of rites above 5,000 m required considerable logistical support and can safely be assumed to have been under central imperial control. In one case, the Spanish chronicler, Hernández de Príncipe reports the final mountaintop words of a 10-year-old Incan girl victim, Tanta Carhua, as "[f]inish with me now, because the celebrations they held for me in Cuzco were enough" (1, 12). It seems likely that all such children had to be present in the capital at some stage in a ritual process that could have extended over a significant period.

Archaeology and written accounts support a view of the regular redistribution of both people and goods via the sophisticated transport network of roads and way stations that eventually incorporated a territory of 10^6 km^2 (13), and there is *prima facie* evidence of connections between high-altitude ritual sites and other regions of the empire. For example, Lightning Girl was accompanied by vessels produced in the Cuzco and Lake Titicaca regions (14), whereas food stuffs found with all three Llullaillaco children mainly came from the lowlands and included maize and peanuts, as well as the drug, coca (*Erythroxylon*

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[l]Excluding the human and material remains of modern mountaineering, such as the 1924 Everest expedition.

[m]"Llullaillaco Maiden" is a conventional name and does not necessarily denote anything concerning her sexual or reproductive status at death.

[n]"High resolution" distinguishes diachronic "lifeways information" provided by hair segmental analysis from bulk isotope measurements of bone collagen.

[o]Within the last 150 years, eruptions of Volcán Llullaillaco occurred in 1854, 1868, and 1877 (www.volcanolive.com/llullaillaco.html).

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coca). Each child was clothed and variously adorned with metal diadems, feathered headdresses, necklaces, and bracelets. Further high-status artifacts from different parts of the empire included small anthropomorphic and camelid figurines in gold, silver, and coastal spondylus shell, as well as textiles, feather items, ceramics, wooden vessels, and slings (4).

The combined historical and archaeological data suggest that victims chosen for sacrifice may have traveled over considerable geographical distances and experienced significant altitudinal changes during their final months of life. The provision of food for the children may thus have been to sustain them in the structure in which they were placed, in the presence of the god. Such offerings are similar to those made to the cult mummies (*mallqui*) of the Inca descent groups (*ayllus*) that were set up in temples (*huacas*) (15). Betanzos, in 1557 (16), and Hernández Principe, in 1621 (17), said that the sons and daughters of local rulers might be ritually killed, and other children could be given as tribute to the centre by local communities (4). One special category were the *acllas* (“chosen women,” “virgins of the sun,” or “virgins of the Inca”), who were selected from around the age of four to live under the guardianship of priestesses and who, at approximately age 14, would either be given to local nobles as wives, consecrated as priestesses, or offered as human sacrifices during state *capacocha* ceremonies (4, 18).

Advances in the chemical analysis of archaeological hair, including validation of mtDNA evidence (19), serial isotopic measurements (20–22), and drug testing (23), offer valuable avenues of archaeological investigation. Although the established use of stable isotopes in archaeology as diet and locational indicators frequently uses bone collagen or tooth enamel, bone is constantly remodeled throughout life, presenting an averaged dietary measurement, whereas tooth enamel represents the childhood signature. In contrast, scalp hair grows 1 cm a month on average in most human populations and, once formed, does not undergo further biogenic alteration (24).

Stable isotopes of carbon, nitrogen, oxygen, hydrogen, and sulfur in the tissues of an organism provide an indication of the conditions and diet at the time of formation. Stable carbon isotopes can distinguish marine from terrestrial dietary protein and plants according to the photosynthetic pathway they use. C_3 plants, using the Calvin–Benson photosynthetic pathway (most grasses, trees, roots, and tubers) (25), fractionate carbon differently from C_4 plants (such as maize), which use the Hatch–Slack pathway (26) and are restricted to growing at elevations below $\approx$2,500 m; variations in $^{13}C/^{12}C$ isotopic ratios distinguish C_3 from C_4 plants in the tissues of humans and animals (27). Nitrogen isotopes can successfully distinguish plant from animal protein and thus define trophic level, the position that an organism occupies in the food chain (28). Oxygen and hydrogen isotopes are known to vary locally with meteoric water and are thus assumed to track differences in temperature and altitude (29). Sulfur isotopes can discriminate between marine and terrestrial diets and can track changes in diet where foodstuffs of varying δ-^{34}S content were sourced from different background geologies (30). Because there is only weak fractionation between diet and body protein, sulfur can be considered as a type of geolocation indicator. By using these forms of analysis, serial segments along any hair shaft can yield diachronic information for the final months and years of a life history. By equating distance along hair strands with months before death (10 mm $\approx$ 1 month), we may track isotopic dietary change over time from the proximal root.

Results

The calibrated accelerator mass spectrometer radiocarbon date estimate for the Llullaillaco Maiden indicates that she died in the period A.D. 1430–1520.[p] We assume the other child sacrifices also relate to the preconquest period, during the time of the rapid Inca imperial expansion.

At the time scale of the individual victims' lives, dietary isotopic data are seen to vary. In the case of the Maiden, both δ-^{13}C and δ-^{15}N in the scalp hair change dramatically $\approx$1 year

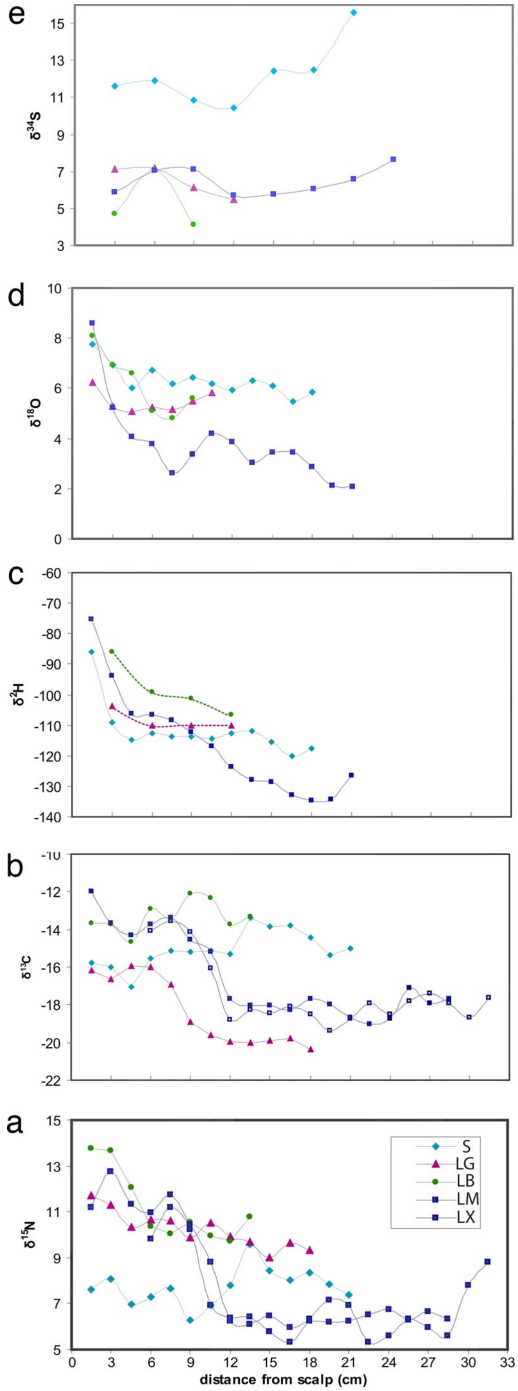

Fig. 1. Serial isotopic data from scalp hair taken from each child. S, Sara Sara Sarita; LG, Llullaillaco Lightning Girl; LB, Llullaillaco Boy; LM, Llullaillaco Maiden; LX, cut hair found with the Llullaillaco Maiden. (*a*) δ-$^{15}N_{AIR}$. (*b*) δ-$^{13}C_{V\text{-}PDB}$. (*c*) δ-$^{2}H_{V\text{-}SMOW}$. (*d*) δ-$^{18}O_{V\text{-}SMOW}$. (*e*) δ-$^{34}S_{V\text{-}CDT}$.

[p] OxA-14878: 400 ± 25, calibrated using Oxcal version 3.10 and the INTCAL04 data set at 95.4% probability 1430 A.D. (82.3%) 1520 A.D. and 1590 A.D. (13.1%)1620 A.D.

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Table 1. Summary of DNA sequence data

		HVR1						SNPs							
		16189	16223	16293	16325	16327	16362	12705	5178	10398	10400	7196	4715	4491	Haplogroup
DNA extract	Cambridge Reference Sequence	t	c	a	t	c	t	c	c	a	c	c	a	g	
Tg701 L01 – Boy	L16209/H16356		t	.	c	t									
	L16055/H16410	c	t	.	c	t	.	t	.	g	t	a	g	.	C
	L16209/H16356 (UCL)														
Tg707 L07 - Boy (hair in bag)	L16209/H16356		t	.	c	t									
	L16055/H16410	c	t	.	c	t	.	t							(C)
	L16209/H16356 (UCL)		t	.	c	t									
Tg703 L03 - Lightning girl	L16209/H16356		t	g	c	.									
	L16055/H16410	.	t	g	c	.	c		a	g	t	.	.	.	D
	L16209/H16356 (UCL)														
Tg708 L08 - Lightning girl (hair in bag)	L16209/H16356		t	g	c	.									
	L16055/H16410														(D)
	L16209/H16356 (UCL)		t	g	c	.									
Tg704 L04 – Maiden	L16209/H16356		t	.	c	.									
	L16055/H16410	.	t	.	c	.	c	t	a	g	t	.	.	.	D
	L16209/H16356 (UCL)		t	.	c	.									
Tg705 L05 - Maiden (hair in 1st bag)	L16209/H16356		t	.	c	.									
	L16055/H16410	.	t	.	c	.	c	t		g	t				(D)
	L16209/H16356 (UCL)		t	.	c	.									
Tg706 L06 - Maiden (hair in 2nd bag)	L16209/H16356		t	.	c	.									
	L16055/H16410							t		g	t				(D)
	L16209/H16356 (UCL)		t	.	c	.									
								N*/R*	D/M*	N*/L3	M*/L3	M*/CZ	M/CZ	M/M9	

Column "HVR1 Primers" lists the primer pairs.

Haplogroups segregated by SNPs (target SNPs provided courtesy of Vincent Macaulay, University of Glasgow) N*/R* D/M* N*/L3 M*/L3 M*/CZ M/CZ M/M9. HVR1 sequences labeled UCL were independently extracted and amplified at University College London (UCL) by I.B.

before death (Fig. 1 *a* and *b*) as her diet became suddenly rich in animal protein and C_4 plants. Carbon values (Fig. 1*b*) for the final 4.5 months, imply increasing reliance on C_4 foodstuffs during the final pilgrimage to the mountain. Nitrogen isotopes (Fig. 1*b*) show a dramatic (an almost 5‰ increase) shift to more positive values [i.e., a magnitude greater than an average trophic level shift (≈3–4‰) (21)] at 12 months before death and likely indicate increased meat consumption subsequent to a meat-poor (presumed peasant) diet.

The other two Llullaillaco individuals also show increasing nitrogen values over the time frame represented, whereas Sarita shows variation more typical of seasonal change. The Llullaillaco Boy (with a little over a year's hair growth) already had a C_4-rich diet for the recorded time frame, with some evidence for seasonal differences within his diet. It is of course possible that the boy's diet could have changed more significantly before this. In contrast to the Llullaillaco individuals, Sarita's hair shows an overall moderate decrease in C_4 contribution to her diet near to death.

Hydrogen (δ-^{2}H) and oxygen (δ-^{18}O) isotopes (Figs. 1 *c* and *d*) show little variation and then shift rapidly before death in all individuals. The Maiden's hair showed increased δ-^{18}O and δ-^{2}H values at ≈4.5 cm (4.1‰ and −106.1‰ to 8.6‰ and −75.3‰, respectively). The Llullaillaco sulfur (δ-^{34}S) data shows little diachronic variation and ranges from 4.1‰ to 7.7‰; Sarita, by contrast, shows a 5.2‰ decrease in δ-^{34}S.

All four individuals from Llullaillaco and Sara Sara were found with small bags containing cut hair. Both the DNA and isotopic data demonstrate that the hairs from each accompanying bag matched either its owner or a close maternal relative. The mtDNA sequence data, obtained from all samples except Sarita [Table 1 and supporting information (SI)], discounts any close maternal relationship between the three Llullaillaco children.[q] Partial HVR1 sequences generated independently in a second laboratory matched the initial sequences exactly, confirming the recovery of authentic mtDNA. Confirmation of the haplogroups was obtained through the cloning and sequencing of seven mtDNA coding region SNPs, which in all cases confirmed the initial findings. Whereas the HVR1 sequences place the mtDNA lineage of the two female Llullaillaco mummies within mitochondrial haplotype D, the boy belongs to mitochondrial haplotype C.

We also were able to diachronically match the isotopic data for the Llullaillaco Maiden and the bagged cut hair strands accompanying her, demonstrating that her bagged hair represents hair cut at 6 months before death (Fig. 1 *a* and *b*) and that her hair was trimmed and elaborately rebraided immediately before death.

The Llullaillaco scalp hair analyses show very high levels of the coca metabolite benzoylecgonine. The Maiden registers at ≈3.5 times higher than for any other South American mummy previously analyzed (Llullaillaco Maiden, 1,803.9 ng per 10 mg of hair; Llullaillaco Boy, 493.7 ng per 10 mg of hair; Llullaillaco Girl, 127.9 ng per 10 mg of hair).

Discussion

Our isotopic data contrast with the previous interpretation of published results examining the presumed seasonally fluctuating importance of maize in the diets of the Aconcagua boy (22, 31) and the Chuscha girl (32) as a means of establishing season of death (22, 31). Reexamining the isotopic data, we consider that only the diet of the Aconcagua boy is potentially explicable as due to season, with death occurring at a time when δ-^{13}C was more negative, i.e., at the beginning of a period of low maize consumption, possibly in early autumn within the Southern Hemisphere (April/May) (31). However, judging from carbon isotope data, the Aconcagua boy had a C_4-rich diet throughout the period of almost 2 years before death, represented by his surviving hair. It is therefore hard to sustain the inference that death occurred at a time of low maize consumption, although the nitrogen signature does suggest protein depletion (22).

Our results matching scalp hair with bagged hair modify or amplify historical information. Our data are ampliative in that

[q]McKenney, K., Rasmussen, E. M., Castaneda, J., Fourth World Congress of Mummy Studies, Sept. 4–10, 2001, Nuuk, Greenland.

we know from José de Arriaga (33) that a child's hair was kept after it was first cut at age 4–5 (1), with the implication that this was a symbolically loaded ritual. The data are modifying in that the Maiden's retained cut hair represents a later event unrecorded by chroniclers. Hair-cutting is cross-culturally associated with status change, itself typically involving rites of separation (often symbolized by acts that sever something), transition (liminal rites), and, finally, incorporation (34). Cutting the Maiden's hair 6 months before death could symbolize separation from the life phase that she entered 1 year before her death, when her status was raised. The final trimming proximal to death and the elaborate braiding presage the Maiden's ultimate separation from the living world. Bearing in mind the importance of the sun and the solar year in elite Incan cosmology, the intervals of 12 and 6 months in the time-resolved data obtained from serial sections of the Maiden's hair, albeit inferred from average hair-growth rates, may not be coincidental. The supposition is congruent with the forward planning evidenced by the dietary shift, which carries the implication that, through the social elevation of the Maiden, a countdown to sacrifice had begun.

Volcán Llullaillaco is in an area first brought under imperial control in the 1470s. On the assumption that each individual likely made their final pilgrimage from the Imperial capital at Cuzco, ≈1,420 km from Llullaillaco and ≈300 km from Sara Sara (shortest distance by road), the journey time must be measured in months. Given the distance that can be walked in a day by a loaded llama and the normal spacing of way stations along the Inca road is 20 km, we can envisage a minimum journey time to Llullaillaco of ≈2.5 months and a minimum journey time to Sara Sara of just over 2 weeks. However, assuming a sizeable retinue for the pilgrimage and allowing for various rites to be performed at stations en route, it is likely that the journeys took longer, a supposition supported by the isotopic data.

For Llullaillaco, the change in trend of carbon, hydrogen, and oxygen isotope ratios (Fig. 1 *b–d*), discernible from ≈4.5 months before death, is consistent with an altered diet increasingly reliant on maize, which was likely stored in way stations. However, the trend toward heavier isotopic values coincident with the montane ascent is, at first sight, puzzling, because water from progressively higher altitude up to 5,000 m should produce the reverse trend (35). A small percentage of the bound water in human hair keratin is biochemically and isotopically derived from drinking water, whereas the bulk is considered to be derived from water inherent in food (22). Consequently, the use of lowland maize and locally sourced water or old snow that may have undergone natural freeze–thaw/evaporation cycles (36) for rehydrating and simmering the maize would be expected to concentrate the heavier oxygen and hydrogen isotopes through evaporation during cooking at altitude.

Although δ-^{2}H, δ-^{18}O, and δ-^{34}S remain developmental isotopic tools in archaeology, by factoring an average fractionation of 17‰ between hair and body water δ-^{2}H, as proposed by Sharp (22), δ-^{2}H values for all four individuals (also backed by the δ-^{18}O data) might indicate a highland (in Incan terms, low status) origin for all four individuals. Using a correlation between altitude and isotopic composition of local meteoric water values derived from the GNIP (Global Network of Isotopes in Precipitation) database for Northern Argentina (37), Sharp (22) suggested that the Aconcagua Boy had lived at an altitude of ≈1,600 m above sea level for the last year of his life. The hydrogen data for the Aconcagua Boy (ranging from −60 to −20‰) suggests that he likely originated from a lower elevation than the three Llullaillaco individuals (ranging from −135 to −75‰). Fluctuating δ-^{18}O and δ-^{2}H values along the distal portion of the Maiden's hair before the significant increase may represent summer/winter variations in environmental water. A similar but weaker trend is seen in δ-^{18}O and δ-^{2}H in the Boy and Sarita at around the same ≈45-cm mark. There is little variation in δ-^{18}O in the hair of the Lightning Girl, with a minor increase in δ-^{2}H near the scalp.

The sulfur data (Fig. 1*e*) do not provide evidence of a marine contribution to diet. With little fractionation between diet and body protein, mean ocean values would be expected to be ≈20‰ (38). Previously published sulfur data for the Aconcagua Boy also show a nonmarine signature (31). The sulfur data from the three Llullaillaco individuals coincide at ≈6 months before death, and may indicate colocation of these individuals at this point, perhaps in Cuzco where all three individuals most probably began their final journeys.

Although we know that Sarita, like the Ampato Maiden (1), received a blow to the head, the cause of death in the three Llullaillaco children has not been definitively established. At an altitude of 6,700 m, there are significant risks of acute mountain sickness, high-altitude pulmonary edema, and high-altitude cerebral edema, even for acclimatized individuals (e.g., >3,000 m) (39, 40). Fray Bernabé Cobo (41) writes specifically of the sacrifice of boys that "[t]hey were killed by strangulation with a cord, or by a blow with a club and then they were buried, and sometimes they got them drunk before having them killed," giving them maize beer (*chicha*) to "dull their senses." Alonso Ramos Gavilán wrote in 1621 that "[w]hen the hour of sacrifice came, they placed in the mouth a fist full of crushed coca leaves with which they smothered [the child]" (42). Although a chewing "quid" of coca leaves was recovered from inside the Maiden's left cheek (3), she was not necessarily killed by it. Coca has a known ameliorative effect on altitude sickness but has a complex and not wholly adaptive action (43), whereas the physiological chemistry of altitude sickness has been subject to considerable recent controversy (44). It is reasonable to conclude that coca use at least inured the children to their situation, thus hastening their deaths (1, 12).

Evidence of stress is clear in the case of the 7-year-old Aconcagua Boy, whose clothes were covered in vomit and diarrhea, features interpreted by de Cicco (45) as indicative of a state of terror. The vomit was stained red by the hallucinogenic drug achiote (*Bixa orrellana*), traces of which were also found in his stomach and feces (46), although his death was likely caused by suffocation, his body apparently having been crushed by his textile wrapping having been drawn so tight that his ribs were crushed and his pelvis dislocated (6).

The mtDNA sequence data for the three Llullaillaco individuals show that they belong to haplogroups C and D, which, along with haplogroups A and B, represent the four major mtDNA lineages found within Amerindian populations that have not undergone admixture with post-Columbian immigrants (47). Little geographic information can be ascertained from the mtDNA results, because both haplogroups are common among many Amerindian populations and a large amount of population specific variation is observed within both haplogroups (47). The apparent southward trend toward higher frequencies of both haplogroups (48), although possibly coincidental, correlate with the calibrated accelerator mass spectrometer radiocarbon date estimate for the Llullaillaco Maiden, which also indicates that she died during the period of the Inca's southern expansion and consolidation and plausibly at or close to the time of incorporation of these territories.

Collectively our data shed light on the nature and phasing of Inca rituals and suggest significant changes of dietary input attributable both to the changing cultural context (status elevation) and natural context (physical elevation) of those chosen for sacrifice. There are many cross-cultural parallels for the social elevation for such victims before their deaths, reflecting perhaps certain prior subideological behaviors that may impede elites from actually sacrificing their own progeny when a proxy could serve as well (34).

Notwithstanding the fact that the ascent of Llullaillaco would have necessitated dietary change proximal to death in terms of

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increasing dependence on the rehydration of appropriate dried foods, such as maize and charki, the dramatic increase in protein in the Maiden's diet, inferred from nitrogen, 1 year before her death is most plausibly attributable to status change. Diet and status were strictly correlated in the Inca empire (49); thus, the in-step shift in carbon values, consistent with an increased intake of C_4 foodstuffs, such as the historically attested "elite" food, maize (16), can be taken to indicate that the Maiden had been raised in status, presumably for the express purpose of making her an appropriate sacrifice.

The logistical effort involved in the ritualized killings of children at high peaks was unprecedented and presumably designed to inspire awe and instill fear. The placement of the *capacocha* children could act on two levels: first, that of a sophisticated belief system in which the existence of gods was not in doubt; and, second, on a more atavistic level, as a successful operationalization of Machiavelli's insight that fear coupled with respect is the most effective tool of governance within a state system (50) and that local rulers bought into what D'Altroy terms a "territorial-hegemonic" model (51). In these terms, the *capacocha* children could have functioned in a way somewhat similar to the clan cult mummies in the temples but set up on the high peaks by the imperial authorities to attract devotion from a much wider constituency. In this role, they may have been conceptualized as functioning in different ways according to need and season, appeasing the destructive forces of the mountain gods and ensuring goodwill from the weather god, Illapa, and/or the regional weather deities thought to reside in mountains.

The data presented here show how an interdisciplinary approach can yield insights into rituals unrecorded in the chronicles but consonant with the broader understandings of cosmology that they have provided to date.

Materials and Methods

Documentation of the Inca structures on Llullaillaco in 1983–1985 led to significant management concerns. In particular, looters' pits on the summit and elsewhere on the mountain, where protection is logistically unfeasible, informed the decision by the Cultural Patrimony Office of the Secretary of Culture of Salta, Argentina, for excavation to proceed (ref. 1, p. 258ff). In 1999, a small team of archaeologists and anthropologists, including students and native indigenous Quechua-speaking mountaineers, surveyed and excavated the summit subcomplex consisting of shelters, cairns, and an artificial platform, which extended $\approx$100 m along a spur running northward from the absolute high point (p. 16ff and figures 17–19b and photograph 13a in ref. 52; figures 3–15 in ref. 53). The sacrificial platform consisted of a revetted dry-stone structure with an internal, offset circular stone setting; it contains three tombs and several niches for offerings, which were documented *in situ* by using standard three-dimensional recording protocols (appendices B–E and figures 20–24b in ref. 52; p. 49ff and figures 16–18 in ref. 53). Finds were removed to a dedicated laboratory and storage facility at Catholic University in Salta and, since 2004, have been curated in the new Museo de Arqueologia de Alta Montaña (52, 53). The unique status of these finds in the cultural heritage of pre-Hispanic Andean peoples necessitates the use of analytical techniques with minimal destructive impact, such as those reported here. Analysis of strontium 87/86 ratios (54) was ruled out, because the necessary destructive sampling would also require thawing the Llullaillaco bodies.

Sampling protocol varied according to the different condition of the remains: Sarita's hair was loose on her cranium because her scalp tissue had largely decomposed, whereas the hair from the Llullaillaco children, placed >1,000 m higher, was still embedded in frozen scalp tissue and had to be cut at the proximal end with surgical scissors to avoid damage. Loose hair was sampled from the small accompanying bags through extant small splits. An accelerator mass spectrometer radiocarbon date estimate was obtained for scalp hair from the Llullaillaco Maiden prepared according to standard protocols at the Oxford University (Oxford, U.K.) radiocarbon accelerator unit.

Scalp hair samples were prepared for isotopic analysis by using standard protocols (55, 56). Samples were soaked overnight in 1:2 (vol/vol) chloroform/methanol, sonicated and rinsed three times in deionized water before lyophilization. Each shaft was oriented and cut into 15-mm serial segments (where insufficient sample existed, 30-mm serial segments were used), working from the proximal end. Samples (individual or paired-fiber segments, dependent on mass) and internal standards were weighed into tin capsules for carbon and nitrogen isotope analyses, conducted by using a ThermoFinnigan Delta plus XP mass spectrometer and elemental analyzer (EA/IRMS, elemental analyzer/isotopic ratio mass spectrometer). Samples and standards for oxygen and hydrogen isotope analyses were weighed into silver capsules, equilibrated to ambient laboratory conditions, and analyzed with a ThermoFinnigan Delta plus XP mass spectrometer and high temperature pyrolysis reduction system (TC/EA, high temperature conversion/elemental analyzer). Samples for sulfur analysis were weighed into tin capsules with 3 mg of vanadium pentoxide and analyzed at IsoAnalytical (Cheshire, U.K.) with an elemental analyzer/isotopic ratio mass spectrometer. Data are reported in the conventional delta notation relative to internationally recognized standards: Pee Dee Belemnite (δ-$^{13}C_{V\text{-}PDB}$) for carbon; air (δ-$^{15}N_{AIR}$) for nitrogen; standard mean ocean water (δ-$^{18}O_{V\text{-}SMOW}$) for both oxygen and (δ-$^{2}H_{V\text{-}SMOW}$) hydrogen (57); and Canyon Diablo Troilite (δ-$^{34}S_{V\text{-}CDT}$) for sulfur. Because of the potential exchangeable nature of hydrogen and oxygen within hair (58), repeat measurements were performed at two institutions; the same values and trends were observed for both sample series. Precision data are reported in SI. The integrity of sample preservation for each individual was confirmed by histology and carbon-to-nitrogen atomic ratio.

Eight hair samples relating to the four individuals were subjected to mtDNA analyses using strict ancient DNA protocols designed to prevent contamination (19) to both confirm the mtDNA haplogroup of the children and to investigate the relationship between the bagged samples and the Llullaillaco children. Three samples were scalp hair from the Llullaillaco children (L01, L03, and L04), with one from Sarita. Four additional samples came from the bags accompanying the Llullaillaco children (L05–L08). Five samples (L04–L08) were subject to mtDNA extractions by two independent laboratories; the remainder (L01 and L03) were investigated at one laboratory because of limited material. Samples (5–10 cm) were decontaminated, and mtDNA was extracted according to previously published protocols (19, 59), PCR-amplified, cloned, and sequenced from the mitochondrial Hypervariable I (HVR1) region. Further mtDNA coding region SNPs were PCR-amplified, cloned, and sequenced to confirm the mitochondrial haplogroups of the specimens. Full details of PCR primers and mtDNA regions amplified are found in SI.

Coca metabolites were investigated by using portions of cleaned hair vortex-mixed in test tubes with 1 ml of 0.1 M HCl. The mixture was then extracted overnight in a 45°C water bath. The acid extract was neutralized with 0.1 ml of 0.1 M NaOH, buffered with 0.9 ml of phosphate buffer to a pH value of $\approx$7.2, and then vortex-mixed and spun for 10 min at 640 $\times$ g. The extract was removed from the hair and a second spin performed. Twenty-five microliters of extract was analyzed by an RIA (Diagnostic Products, Los Angeles, CA) according to the manufacturer's recommendations for the determination of the cocaine metabolite benzoylecgonine in urine. A result of >5 ng metabolite per 10 mg of hair was interpreted as positive. Negative control hair was supplied by laboratory personnel.

Positive control hair came from Oklahoma Medical Examiner files of known chronic cocaine users.

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