Tracing the geographical origin of cocaine

Cocaine carries a chemical fingerprint from the region where the coca was grown.

Here we show that cocaine originating from different geographic regions in South America can be identified by its isotope-ratio signature. The distinct carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope-ratio combinations allow the country of origin to be determined for the principal coca-growing regions along the Andean Ridge. By combining this information with detectable differences in the patterns of the trace alkaloids truxilline and trimethoxycocaine, we correctly identified the source of 96% of 200 cocaine samples.

Cocaine is the most widely used narcotic drug, making the determination of the geographic origin of illicit cocaine the focus of intense investigation by the forensic community[1,2]. Previous studies have concentrated on detecting the trace residues present in cocaine or the trace alkaloids that are extracted with it; these have met with limited success, although they have been valuable in identifying the processing methods peculiar to different regions. However, refined cocaine base is often transported from one country to another for its final conversion to cocaine hydrochloride, making the source harder to identify.

Stable-isotope ratios (δ) have been used as indicators of the geographical source of a wide variety of biological and non-biological materials, from migrating butterflies[3] to emeralds[4]. The natural isotopic variation in organic matter in plants can be applied to predict the variation in $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values along ecological gradients[5–8].

There are large environmental differences in the coca-growing regions of South America. We investigated the variation in $\delta^{13}C$ and $\delta^{15}N$ of coca leaves and of the extracted cocaine from plants from different countries along the Andean Ridge, collecting 200 coca leaf sets from throughout each of the five primary growing regions: the Chapare Valley of Bolivia, the Huallaga and Ucayali Valleys and Apurimac Valley of Peru, and the Putumayo–Caqueta and Guaviare regions of Colombia (Fig. 1). Subsets of the same coca leaves were then extracted for determination of total alkaloids[9], and trimethoxycocaine and truxilline content[10]. We purified cocaine base (to over 98% purity) from these extracts by column chromatography on alumina and measured $\delta^{13}C$ and $\delta^{15}N$ to a precision of greater than 0.1 part per thousand on corresponding sets of coca leaves and purified cocaine samples[11].

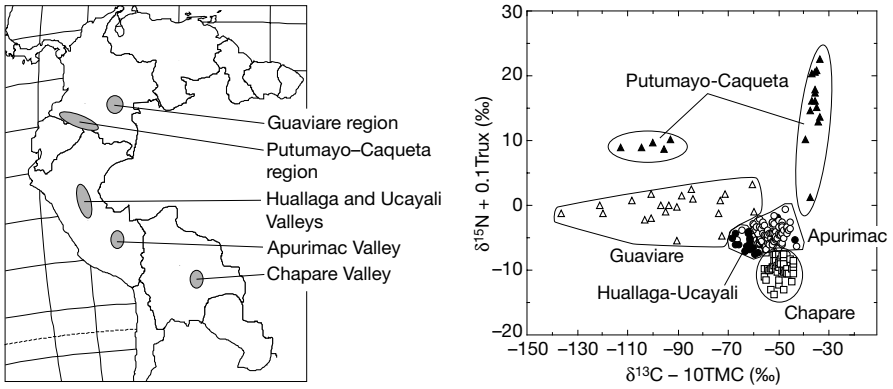

Figure 1 Identification of geographic regions in South America where coca is commonly grown. Left, regions producing illicit cocaine; right, identification of cocaine-growing regions based on a combined model derived from carbon- and nitrogen-isotope ratios as well as abundance of minor alkaloid components. Squares, Bolivia; triangles, Colombia; and circles, Peru. Regions within a country are distinguished by black and white symbols. Trux, truxilline; TMC, trimethoxycocaine. Isotope ratios are expressed as $(R_{sample}/R_{standard} - 1) \times 1{,}000‰$, where R is the molar ratio of heavy-to-light stable isotope; standards for carbon and nitrogen are PDB and air, respectively.

Over the entire geographical distribution, $\delta^{13}C$ and $\delta^{15}N$ values for coca leaves vary from $-32.4‰$ to $-25.3‰$ for $\delta^{13}C$, and from 0.1‰ to 13.0‰ for $\delta^{15}N$. Leaves from the Putumayo and Caqueta regions of Colombia are distinguishable from each other by their $\delta^{13}C$ content, as are those from the Huallaga and Ucayali Valleys from the Apurimac Valley of Peru. Coca leaf from Bolivia has consistently less ^{15}N than material from Peru. There is more ^{15}N in coca leaves from Colombia and least in coca grown in the Chapare Valley of Bolivia.

To determine the probability that a sample could have originated from one of these three countries, we used bivariate mean and standard deviation parameters to estimate the frequency with which we could correctly identify the country of origin of these coca-leaf samples. We predicted the country of origin by assigning a sample to the region to which it was associated with the highest probability. Using this approach, we accurately predicted the country of origin of 90% of the 200 coca-leaf samples.

The truxilline and trimethoxycocaine content of coca leaves contributed additional information, showing a clear demarcation between the Guaviare and Putumayo–Caqueta regions and locations in Peru and Bolivia (Table 1). Although it was not possible to differentiate Peruvian and Bolivian cocaine from their trace-alkaloid analyses, these samples were distinguishable by $\delta^{13}C$ and $\delta^{15}N$ analysis.

Combining alkaloid and isotopic-ratio analyses is a powerful means for determining the country of origin of cocaine from these different regions in South America (Fig. 1). Taking advantage of our observation that truxilline content and cocaine $\delta^{15}N$ are positively correlated and that cocaine $\delta^{13}C$ and trimethoxycocaine are negatively correlated, a combination plot reveals that the cocaine samples from different regions cluster into tighter groups based on their country of origin. Using our analytical approach, we were able accurately to predict the country of origin of 96% of the 200 cocaine samples.

The environmental basis for this regional variation in coca-leaf and cocaine stable isotopes may be due to differences in soils affecting $\delta^{15}N$, and in the humidity and length of the wet season affecting $\delta^{13}C$. It has been proposed[8] that nutrient cycles in tropical forest ecosystems (typical of coca-growing regions) are more open than in drier, more temperate regions, and the range of coca-leaf $\delta^{15}N$ values are consistent with this idea. $\delta^{13}C$ differences are narrower, but are consistent with patterns predicted by $\delta^{13}C$ theory for plants[5,6].

Table 1 Differences in trace alkaloid amounts among coca populations

Region	*Truxillines*	*Trimethoxycocaines*	*Ratio*
Chapare Valley, Bolivia	2.79 ± 0.41	0.16 ± 0.03	17.2
Huallaga/Ucayali Valleys, Peru	3.80 ± 0.76	0.19 ± 0.04	20.0
Apurimac Valley, Peru	4.11 ± 1.59	0.27 ± 0.06	15.2
Guaviare region, Colombia	4.94 ± 2.20	0.61 ± 0.06	8.1
Putumayo–Caqueta region, Colombia	14.66 ± 4.29	0.20 ± 0.29	7,330

Means and standard deviations of truxilline and trimethoxycocaine content of coca leaves, together with the ratio of truxillines to trimethoxycocaines growing in different regions of South America are shown. Data are given on a w/w% basis using structurally related internal standards.

Tracing the country of origin of cocaine is now feasible through automated, routine analysis of both stable isotopes and trace alkaloids, opening up strategic options for identifying source regions and trafficking routes. We have shown how ecological and isotopic-fractionation principles used to predict isotopic-ratio patterns associated with plants from different ecosystems can also be applied to determine the distribution of an illegal drug, as well as to identify new coca-producing regions as they develop.

James R. Ehleringer*, John F. Casale†, Michael J. Lott*, Valerie L. Ford†

**Stable Isotope Ratio Facility for Environmental Research, Department of Biology, University of Utah, 257 S 1400 E, Salt Lake City, Utah 84112-0840, USA*

e-mail: ehleringer@biology.utah.edu

†Special Testing and Research Laboratory, Drug Enforcement Administration, US Department of Justice, 7704 Old Springhouse Road, McLean, Virginia 22102-3494, USA

1. Moore, J. M. & Casale, J. F. *Forensic Sci. Rev.* **10,** 13–46 (1998).
2. Moore, J. M. & Casale, J. F. *J. Chromatogr. A* **674,** 165–205 (1994).
3. Wassenaar, L. I. & Hobson, K. A. *Proc. Natl Acad. Sci. USA* **95,** 15436–15439 (1998).
4. Giulani, G. *et al. Science* **287,** 631–633 (2000).
5. Farquhar, G. D., Ehleringer, J. R. & Hubick, K. T. *Annu. Rev. Plant Physiol. Mol. Biol.* **40,** 503–537 (1989).
6. Ehleringer, J. R., Hall, A. E. & Farquhar, G. D. (eds) *Stable Isotopes and Plant Carbon/Water Relations* (Academic, San Diego, 1993).
7. Roden, J. S., Lin, G. & Ehleringer, J. R. *Geochim. Cosmochim. Acta* **64,** 21–35 (2000).
8. Martinelli, L. A. *et al. Biogeochemistry* **46,** 45–65 (1999).
9. Moore, J. M., Casale, J. F., Klein, R. F., Cooper, D. A. & Lydon, J. *J. Chromatogr. A* **659,** 163–175 (1994).
10. Moore, J. M., Casale, J. F. & Cooper, D. A. *J. Chromatogr. A* **756,** 193–201 (1996).
11. Ehleringer, J. R., Cooper, D. A., Lott, M. J. & Cook, C. S. *Forensic Sci. Intl* **106,** 27–35 (1999).

Archaeology

Detecting milk proteins in ancient pots

Deciding whether to farm cattle for milk or beef was just as complex in the past as it is today. Compared with meat production, dairying is a high-input, high-output, high-risk operation indicative of an intensive, sophisticated economy, but this practice is notoriously difficult to demonstrate in the archaeological record[1]. Here we provide evidence for the presence of milk proteins preserved in prehistoric vessels, which to our knowledge have not been detected before. This finding resolves the controversy that has surrounded dairying on the Scottish Atlantic coast during the Iron Age[2–5] and indicates that farming by the early inhabitants of this harsh, marginal environment was surprisingly well developed.

The analysis of sorbed lipid residues in prehistoric ceramics has provided a powerful new indicator of how vessels were used[6–8]. Although proteins are more diagnostic of specific foodstuffs than lipids, they are difficult to extract from archaeological ceramics[9]. We have developed an immunological detection method, the digestion-and-capture immunoassay (DACIA)[10], which overcomes this difficulty by dissolving the ceramic then capturing the liberated proteins for immunodetection.

We obtained sherds from nine coarseware cooking vessels, dated to the middle of the first millennium BC, from the fill of an Early Iron Age house at Cladh Hallan, South Uist, in the Outer Hebrides, and analysed them by DACIA. Extracts were tested using a monoclonal antibody raised against heat-degraded and dephosphorylated bovine α-casein (about 1.4% w/v milk), which was specific for bovine milk.

Immunological analysis of archaeological materials has been criticized for the lack of negative controls[11], so we included an extensive array of reference samples (Fig. 1). Seven of nine of the interior sections of sherds recovered from Cladh Hallan tested positive for casein and the amounts were comparable to those found on experimentally buried milk sherds (Fig. 1). DACIA analysis failed to detect the presence of bovine α-casein in the associated sediment or exterior surfaces of the samples.

The large number of neonatal cattle remains found at this site (42% of individuals) has been attributed to the deliberate culling of young calves in order to preserve fodder in an adverse environment[2,3] or to sustain a high-input dairying economy[4,5]. The presence of bovine α-casein on a substantial number of sherds (Fig. 1) lends support to the latter interpretation. Our successful characterization of protein residues after 2,500 years demonstrates the potential of DACIA as a high-resolution technique for determining how archaeological ceramics were used.

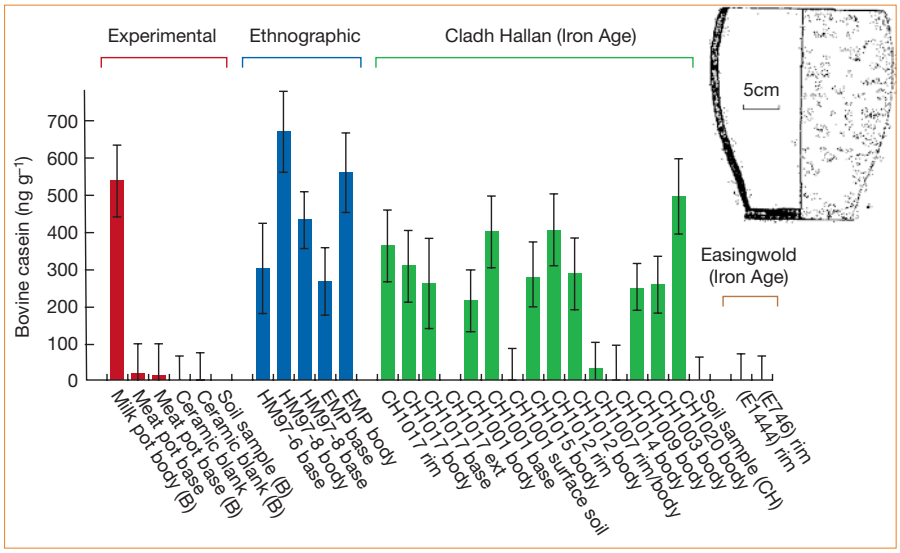

Figure 1 Amounts of bovine α-casein present in samples of pottery and soil, as determined by duplicate assay using digestion-and-capture immunoassay with a monoclonal antibody raised against this protein. Error bars, one standard deviation. The assay is specific only for cows' milk and is able to detect as little as 100 ng protein per g of ceramic (0.1 p.p.m.). Experimental coarseware pots (ceramic 'blank') were used to boil either milk (milk pot) or beef (meat pot) repeatedly and were buried for 1 year in upland soil. Ethnographic pots were obtained from Pakistan (HM) and India (EMP); each had been recently used to prepare dairy products. Cladh Hallan (CH) vessels (inset) were collected from a single site (fill of house 112, South Uist, Outer Hebrides). Domestic cooking pots from Easingwold, North Yorkshire (E), contained large amounts of well-preserved animal fats.

Oliver Craig*, Jacqui Mulville†, Mike Parker Pearson‡, Robert Sokol§, Keith Gelsthorpe§, Rebecca Stacey||, Matthew Collins*

**Fossil Fuels and Environmental Geochemistry, NRG, Drummond Building, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE1 7RU, UK*

e-mail: o.e.craig@ncl.ac.uk

†English Heritage, Oxford University Museum, Parks Road, Oxford OX1 3PW, UK

‡Department of Archaeology and Prehistory, University of Sheffield, Northgate House, West Street, Sheffield S1 4ET, UK

§Regional Blood Transfusion Centre, Longley Lane, Sheffield S5 7JN, UK

||Department of Archaeological Science, University of Bradford, Bradford BD7 1DP, UK

1. Halstead, P. *Anthropozoologica* **27,** 3–20 (1998).
2. McCormick, F. in *Life on the Edge: Human Settlement and Marginality* (eds Mills, C. M. & Coles, G.) 49–53 (Oxbow, Oxford, 1998).
3. Gilmour, S. & Cook, M. *Antiquity* **72,** 327–337 (1998).
4. Parker-Pearson, M., Sharples, N. & Mulville, J. *Antiquity* **70,** 57–67 (1996).
5. Parker-Pearson, M., Sharples, N. & Mulville, J. *Antiquity* **73,** 149–152 (1999).
6. Rottländer, R. C. A. in *Proc. 24th Int. Symp. Archaeometry* 403–405 (Smithsonian Institution, Washington DC, 1986).
7. Evershed, R. P. *World Archaeol.* **25,** 74–93 (1993).
8. Dudd, S. N. & Evershed, R. P. *Science* **282,** 1478–1481 (1998).
9. Evershed, R. P. & Tuross, N. *J. Archaeol. Sci.* **23,** 429–436 (1996).
10. Craig, O. E. & Collins, M. J. *J. Immunol. Meth.* **236,** 89–97 (2000).
11. Tuross, N., Barnes, I. & Potts, R. *J. Archaeol. Sci.* **23,** 289–296 (1996).