ARCHAEOLOGY

Early pre-Hispanic use of indigo blue in Peru

Jeffrey C. Splitstoser,[1]* Tom D. Dillehay,[2] Jan Wouters,[3,4] Ana Claro[5]

10.1126/sciadv.1501623

Archaeological research has identified the use of cultivated cotton (*Gossypium barbadense*) in the ancient Andes dating back to at least 7800 years ago. Because of unusual circumstances of preservation, 6000-year-old cotton fabrics from the Preceramic site of Huaca Prieta on the north coast of Peru retained traces of a blue pigment that was analyzed and positively identified as an indigoid dye (indigotin), making it the earliest known use of indigo in the world, derived most likely from *Indigofera* spp. native to South America. This predates by ~1500 years the earliest reported use of indigo in the Old World, from Fifth Dynasty Egypt [ca. 4400 BP (before present)]. Indigo is one of the most valued and most globally widespread dyes of antiquity and of the present era (it being the blue of blue jeans).

INTRODUCTION

After the arrival of the Spanish in the Americas, few things other than precious gems and metals impressed them more than Inca *cumbi* (fine tapestry cloth), primarily because of its silk-like texture and use of brilliant colors (*1*). The superiority of Andean dyes at the time of the conquest should not be surprising, because they were the result of observation and experimentation carried out by native South Americans over several millennia, representing the apogee of one of the world's oldest and most sophisticated weaving and dyeing traditions. Evidence of the early age and complexity of Andean weaving and dyeing practices comes from cotton textiles decorated with indigo from the Preceramic site of Huaca Prieta on the north coast of Peru, which was occupied between 14,500 and 4000 cal BP (calibrated before present).

Huaca Prieta is a large stone and earthen ceremonial mound that was first excavated in the 1940s by J. Bird (*2*), who established the site's early age [~5302–1933 cal BP; see the study by Dillehay *et al.* (*3*)] and made it famous for producing the oldest known cotton fabrics decorated with recognizable art in the Americas. Although Bird's dates for the first use of cotton were subsequently surpassed (*4*, *5*), recent work has shown that textiles were produced at Huaca Prieta even earlier than previously documented.

The original site for the domestication of cotton, *Gossypium barbadense*, has not been determined; however, it is thought to be somewhere on the north coast of Peru (*6*), where it adapted to the region's arid conditions and grows wild or as an escaped plant. To date, the earliest reported *G. barbadense* is from the Nanchoc Valley in north Peru, dated to around 7800 cal BP (*7*). The continued use of *G. barbadense* for textiles, fish nets, bags, and other materials is documented at numerous other sites dating after 6000–5000 cal BP (*8*–*19*).

The earliest textiles decorated with blue at Huaca Prieta were excavated from stratum 44 in J. Bird's HP-3 trench. Although not decorated with indigo, the earliest cotton yarn is from stratum 52, which is radiocarbon-dated at 6882–6657 cal BP [sample AA82121; see the study by Dillehay *et al.* (*3*)]. The next radiocarbon-dated level is stratum 35 at 5848–5585 cal BP (sample AA86948 on charcoal from an intact hearth), suggesting that the two textiles from stratum 44 are about 6200 to 6000 years old. They are both weft twining [the fabric structure is spaced, regular, two-strand, Z-twist weft twining with Z(2s) warp and weft yarns; wefts usually enclose two warp yarns, although groupings of one and three yarns are present] decorated with warp stripes where natural colored cotton, which is a light tan, contrasts with cotton dyed blue and cotton plied with a bright-white fiber from the stem of a vine locally known as *chivo* (*Asclepiadaceae* sp., a member of the milkweed subfamily). To date, the source of the blue color at Huaca Prieta is unknown. A sample of blue yarn from one of these early blue-striped fabrics, specimen 2009.052.01.B (Fig. 1), and samples of blue yarns from seven other fabrics from Huaca Prieta whose ages range from 6200 to 1500 cal BP, were investigated for the presence of indigoid dyes (see Table 1). All textiles analyzed in Table 1 were associated with thin, continuous, stratigraphically intact floors that were deeply buried in the mound at Huaca Prieta (~10 to 18 m in depth) and without intrusive architecture and other features. Fabrics were dated on the basis of their associations with these floors. For a detailed discussion of the phases at Huaca Prieta and their ages, see the study by Dillehay *et al.* (*3*).

Indigoid dyes may be prepared from different plants, such as those belonging to the *Indigofera*, *Strobilanthes*, *Isatis*, and *Polygonum* genera. All indigoid dyes contain indigotin, the main blue dye component, and indirubin, a structural isomer of indigotin. To date, there are no known features to distinguish between the genera; however, research has shown that, statistically, the proportion of indirubin to indigotin is higher in blue-dyed textiles from China, India, and Latin America as compared to those of European origin, for instance (*20*, *21*). This may be due not only to the indigo-producing plants available or selected but also to the vatting procedure in the dyeing process, which involves creating an alkaline-reducing medium needed to solubilize the indigoid dye.

RESULTS

The presence of an indigoid dye has been firmly indicated in five of eight examined samples (Table 1) representing two plain weaves and three twined textiles. An example of chromatographic analysis that is of particular interest is given in Fig. 2, and the spectral analysis of the chromatographic peaks is shown in Fig. 3; both refer to the sample from specimen 2009.065.01.A. The HPLC (high-performance liquid chromatography) chromatogram of sample 2009.052.01.B&C, the oldest

[1]Department of Anthropology, The George Washington University, 2110 G Street NW, Washington, DC 20052, USA. [2]Department of Anthropology, Vanderbilt University, 124 Garland Hall, Nashville, TN 37235, USA. [3]Neerbroek 54, B-2070 Zwijndrecht, Belgium. [4]University College London, Institute for Sustainable Heritage, London WC1H 0NN, UK. [5]Instituto dos Museus e da Conservação, Departamento de Conservação e Restauro–Laboratório de Têxteis, Rua da Janelas Verdes no. 37, 1249-018, Lisbon, Portugal.
*Corresponding author. Email: jcs@ancientamerica.net

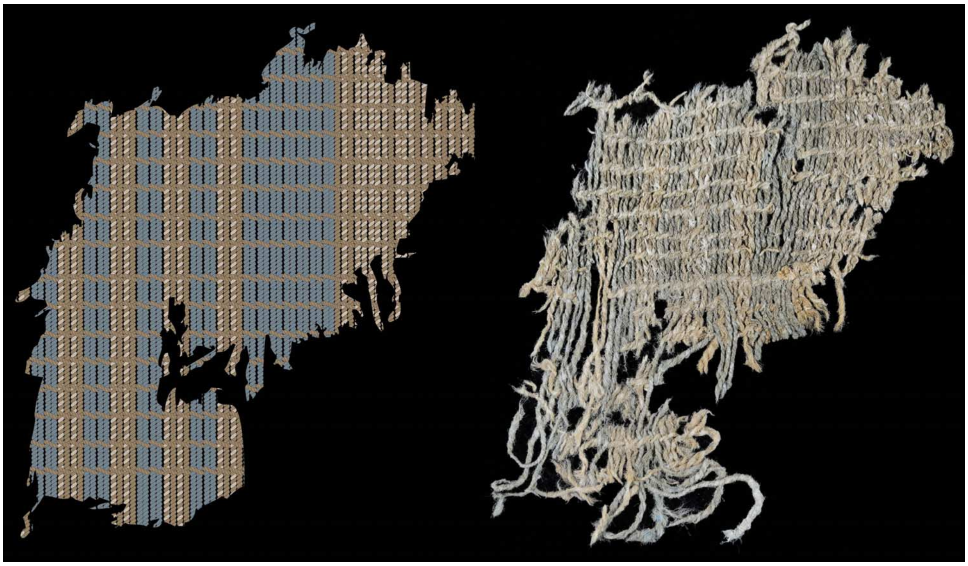

Fig. 1. Diagram and photo of specimen 2009.052.01.B.

Table 1. Huaca Prieta fabrics with blue color that were analyzed, including fabric structure, provenance (level), phase, carbon date, and indigo composition. mAU, milliabsorption units.

Inventory no.	Structure	Level	Phase	^{14}C (2σ cal BP)	Indigo composition
2009.052.01.B&C	Twining	44	3	~6200	25% indigotin, 75% indirubin (0.92 mAU; 288 nm) 59% indigotin, 41% indirubin (0.36 mAU; 600 nm)
2008.038.04	Twining	31	3	~5848–5585	No dye components detected at either wavelength
2008.083.03.C	Twining	32	3	~5848–5585	No dye components detected at either wavelength
2009.028.04.A	Twining	23	4	~5308–4107	No dye components detected at either wavelength
2009.018.01	Twining	19	5	~4107–3455	26% indigotin, 74% indirubin (0.38 mAU; 288 nm) 64% indigotin, 36% indirubin (0.13 mAU; 600 nm)
2009.065.01.A	Plain weave	A2	Cupisnique	~3500–1500	1% indirubin-like, 15% indigotin, 1% indirubin-like, 83% indirubin (1.7 mAU; 288 nm) 1% indirubin-like, 45% indigotin, 1% indirubin-like, 53% indirubin (0.29 mAU; 600 nm)
2009.065.02	Plain weave	A2	Cupisnique	~3500–1500	0% indigotin, 100% indirubin (19.6 mAU; 288 nm) 0% indigotin, 100% indirubin (2.2 mAU; 600 nm)
2008.100.04	Twining	Disturbed	Mixed	Mixed	100% indigotin, 0% indirubin (0.13 mAU; 288 nm) 100% indigotin, 0% indirubin (0.05 mAU; 600 nm)

one of the collection analyzed, as well as the full set of chromatograms and peak integration tables, is included in the Supplementary Materials (section S1). Of particular interest in the analysis of sample 2009.065.01.A is the presence of four dye components. Besides the generally expected indigotin and indirubin, two components that show ultraviolet-visible (UV-vis) spectra closely related to indirubin (Fig. 3, indirubin-like) were observed at trace amounts, but with significantly different retention times. These components have never been reported in other analyses of indigoid dyes worldwide. In three samples, the high relative proportion of indirubin reflects earlier findings on pre-Columbian fabrics (*21*). In one plain weave textile, only indirubin was found. In one sample, 2009.018.01, indigotin and indirubin were observed at trace amounts.

The source of the indigoid dye at Huaca Prieta was most likely *Indigofera* spp.; however, other indigo-producing plants native to the tropics of South America are grown as dye plants today, including *Justicia colorifera* (*cuaja tinta* or *tinta montes*), *Koanophyllon tinctorium* Arruda, and *Cybistax antisyphilitica* Martius (*yangua*). These plants produce blues similar to those of *Indigofera*, and the dye produced by *yangua*, for instance, contains both indigotin and indurbin (*22*). Although

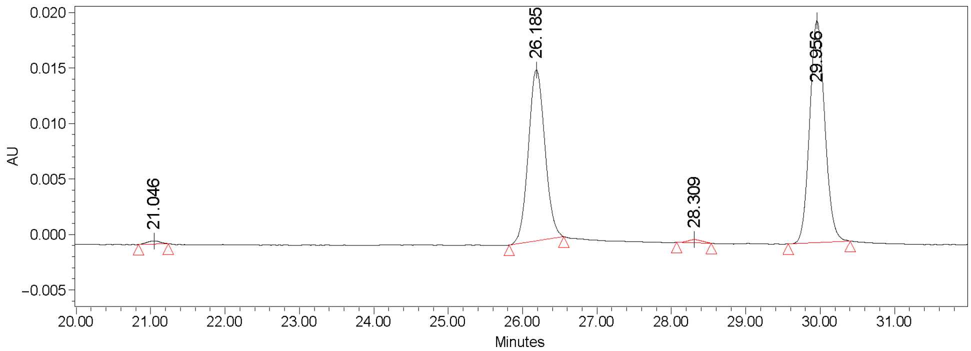

Fig. 2. Chromatographic analysis of sample 2009.065.01.A, recorded at 600 nm.

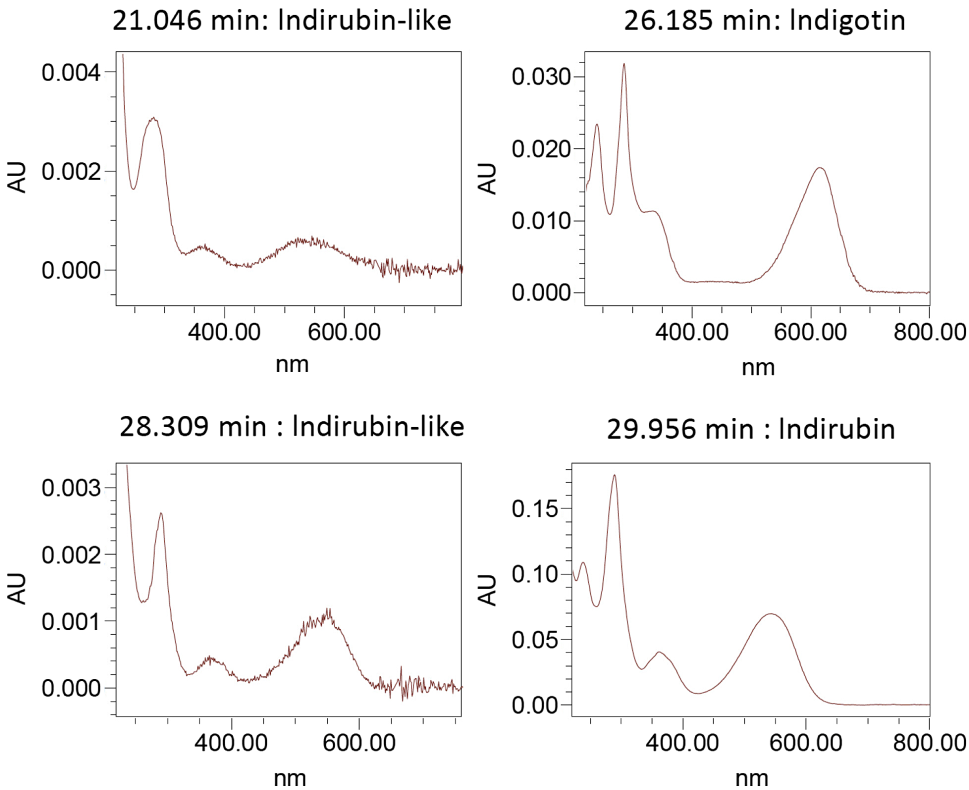

Fig. 3. UV-vis spectral identification of the four peaks from Fig. 2.

these plants are not grown on the north coast of Peru today, their distribution 6000 to 7000 years ago is unknown.

DISCUSSION

Early examples of the use of blue yarns that were most likely colored with indigo are known, but dye analysis had heretofore been unavailable. The composition of the indigoid dyes identified in the fabrics presented here reflects that of earlier findings in Latin American and Asian contexts, in that proportions of indirubin relative to indigotin are significantly higher as compared to European productions. To date, there is no firm evidence to explain these differences, but plant species, harvesting, dye preparation, and actual dyeing, as well as differential conservation processes of essential dye components, may have, alone or in combination, contributed to this observation. One interesting hypothesis, requiring further confirmation, is that ancient vat dyeing technologies favored the formation and uptake by the yarn of indirubin. This would have resulted in a more purplish hue produced by a reddish indirubin and a bluish indigotin. Why no indigoid dye components were found in three of the samples remains an unresolved issue. Limits of peak detectability, too uncertain UV-vis spectral confirmation, sample size, and material losses are all associated with attempts to detect compounds at the limit of detectability. However, the main driver for the present analytical campaign was to see whether indigoid dyes could be detected in textile fragments of the given archaeological context. The conclusion is clear: yes.

To date, the earliest identified blue dyes in the Americas were from Ocucaje and the Paracas Necrópolis dating to around 2500–2250 cal BP (*23*). In the Necrópolis textiles, studies have found combinations of indigo with either *Relbunium ciliatum* (South American madder) or a variety of yellow dyes, to make violet and green, respectively (*21*). In Old World fabrics, there are Egyptian textiles with indigo-dyed bands from the Fifth Dynasty (ca. 4400 BP) (*24*). Early examples of indigo also are known from Xinjiang, China, where indigo has been positively identified as early as 3000 BP (*25*, *26*). It is believed that indigo might have been used earlier in the Near East; however, actual examples with positive identifications of indigo are not available, making the indigo found at Huaca Prieta the earliest known use of indigo.

MATERIALS AND METHODS

All dye analyses were performed with HPLC and photodiode-array detection (HPLC-PDA) (*27*) on yarn extracts prepared with the oxalic acid hydrolysis method (*28*). This method allows for the simultaneous detection of components present in yellow, red, green, blue, and black dyes while avoiding degradation of acid-vulnerable components, such as those bearing glucosidic moities. Analytical results are expressed as a percentage of composition of the components, resulting from the integration of chromatographic peaks at the wavelength indicated. For indigoid dyes, the selected wavelengths were 288 and 600 nm. A full description of sample preparation and HPLC-PDA analysis methods is given in the Supplementary Materials (section S2).

Preceding the indication of the wavelength of detection in Table 1 is the response of the smallest chromatographic peak in milliabsorption units. This helps to evaluate the level of reliability of the analytical result. For acceptance, the peak height should be at least twice as high as the average background noise. This method, which leads to a lower-limit acceptance of peaks than the usual standard, can be used in dye analysis practice because besides a peak's retention time, its UV-vis spectrum must confirm the identification of the component suggested by that retention time. The average background noise is typically 0.025 mAU; thus, a positive detection, pending spectral confirmation, always involves a peak height of at least 0.050 mAU. The identification of individual dye components results from a combination of retention time in the chromatographic system and online UV-vis spectrometry, recorded between 200 and 800 nm, and from comparison of these data with standards. Conclusions are based on chromatographic data obtained at 600 nm, because this wavelength of detection excludes any influence of UV-absorbing noncoloring contaminants in the quantitative estimations of indigoid dye components; however, each analysis was screened for absorptions using the whole detector's wavelength range.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/2/9/e1501623/DC1
section S1. Chromatographic analysis of all samples, recorded at 600 nm.
section S2. Description of sample preparation and HPLC-PDA analysis methods.

REFERENCES AND NOTES

1. B. Cobo, in *Inca Religion and Customs,* R. Hamilton, Ed. (University of Texas Press, Austin, 1990), 279 pp.
2. J. B. Bird, J. Hyslop, *The Preceramic Excavations at the Huaca Prieta, Chicama Valley, Peru. Anthropological Papers* (American Museum of Natural History, New York, 1985), 294 pp.
3. T. D. Dillehay, D. Bonavia, S. Goodbred, M. Pino, V. Vasquez, T. Rosales Tham, W. Conklin, J. Splitstoser, D. Piperno, J. Iriarte, A. Grobman, G. Levi-Lazzaris, D. Moreira, M. Lopéz, T. Tung, A. Titelbaum, J. Verano, J. Adovasio, L. S. Cummings, P. Bearéz, E. Dufour, O. Tombret, M. Ramirez, R. Beavins, L. DeSantis, I. Rey, P. Mink, G. Maggard, T. Franco, Chronology, mound-building and environment at Huaca Prieta, coastal Peru, from 13700 to 4000 years ago. *Antiquity* **86**, 48–70 (2012).
4. J. E. Damp, D. M. Pearsall, Early cotton from coastal Ecuador. *Econ. Bot.* **48**, 163–165 (1994).
5. T. D. Dillehay, J. Rossen, T. C. Andres, D. E. Williams, Preceramic adoption of peanut, squash, and cotton in northern Peru. *Science* **316**, 1890–1893 (2007).
6. D. Piperno, in *From Foraging to Farming in the Andes: New Perspectives on Food Production and Social Organization*, T. D. Dillehay, Ed. (Cambridge Univ. Press, New York, 2011), pp. 275–284.
7. J. Rossen, in *From Foraging to Farming in the Andes: New Perspectives on Food Production and Social Organization*, T. D. Dillehay, Ed. (Cambridge Univ. Press, New York, 2011), pp. 177–192.
8. M. N. Cohen, Archaeological plant remains from the central coast of Peru. *Ñawpa Pacha* **16**, 23–50 (1978).
9. F. A. Engel, in *Antiguo Peru: Espacio y Tiempo: Trabajos Presentados a la Semana de Arqueologia Peruana (9–14 de noviembre de 1959)*, R. Matos Mendieta, Ed. (Libreria-Editorial Juan Mejia Baca, Lima, 1960), pp. 119–128.
10. F. Engel, Un groupe humain datant de 5000 ans a Paracas, Pérou. *J. Soc. Am.* **49**, 7–35 (1960).
11. R. A. Feldman, Life in Ancient Perú. *Field Mus. Nat. Hist. Bull.* **48**, 12–17 (1977).
12. R. A. Feldman, Informe preliminar sobre excavaciones en Aspero, Peru, y sus implicaciones teóricas. *Investig. Arqueológ.* **2**, 20–27 (1978).
13. R. A. Feldman, thesis, Harvard University, Cambridge (1980).
14. A. H. Gayton, Textiles from Hacha, Peru. *Ñawpa Pacha* **5**, 1–13 (1967).
15. M. E. Moseley, G. R. Willey, Aspero, Peru: A reexamination of the site and its implications. *Am. Antiquity* **38**, 452–468 (1973).
16. S. G. Pozorski, thesis, University of Texas, Austin (1976).
17. M. A. Towle, *The Ethnobotany of Pre-Columbian Peru*, S. Tax, Ed. (Aldine Publishing Company, Chicago, 1961), vol. 30, 180 pp.
18. D. Ugent, S. Pozorski, T. Pozorski, New evidence for the ancient cultivation of *Canna edulis* in Peru. *Econ. Bot.* **38**, 417–432 (1984).
19. W. E. Wendt, in *Lecturas en Arqueología* (Museo de Arqueología y Etnología de la Universidad Nacional Mayor de San Marcos, Lima, 1976).
20. J. Wouters, in *Dyes in History and Archaeology Number 12: Papers Presented at the 12th Annual Meeting, Brussels, 1993* (Textile Research Associates, York, England, 1994), pp. 12–22.
21. J. Wouters, N. Rosario-Chirinos, Dye analysis of pre-Columbian Peruvian textiles with high-performance liquid chromatography and diode-array detection. *J. Am. Inst. Conserv.* **31**, 237–255 (1992).
22. D. Cardon, *Natural Dyes: Sources, Tradition, Technology and Science* (Archetype Publications, London, 2007).
23. A. P. Rowe, L. A. Meisch, in *Weaving and Dyeing in Highland Ecuador*, A. P. Rowe, Ed. (University of Texas Press, Austin, 2007), pp. 254–266.
24. J. Balfour-Paul, *Indigo* (Archetype Publications, London, 2006), 264 pp.
25. X. Zhang, I. Good, R. Laursen, Characterization of dyestuffs in ancient textiles from Xinjiang. *J. Archaeol. Sci.* **35**, 1095–1103 (2008).
26. A. Kramell, X. Li, R. Csuk, M. Wagner, T. Goslar, P. E. Tarasov, N. Kreusel, R. Kluge, C.-H. Wunderlich, Dyes of late Bronze Age textile clothes and accessories from the Yanghai archaeological site, Turfan, China: Determination of the fibers, color analysis and dating. *Quatern. Int.* **348**, 214–223 (2014).
27. J. Wouters, in *Scientific Methods and Cultural Heritage: An Introduction to the Application of Materials Science to Archaeometry and Conservation Science,* G. Artioli, Ed. (Oxford Univ. Press, New York, 2010), pp. 410–413.
28. J. Wouters, C. M. Grzywacz, A. Claro, A comparative investigation of hydrolysis methods to analyze natural organic dyes by HPLC-PDA - nine methods, twelve biological sources, ten dye classes, dyed yarns, pigments and paints. *Stud. Conserv.* **56**, 231–249 (2011).

Acknowledgments: We thank the Ministerio de Cultura, Lima, Peru, for granting us permission to work at Huaca Prieta. We are grateful to C. Galvéz and J. Briceño (Dirección Regional del Ministerio de la Cultura, Trujillo) for their support. **Funding:** This project was financed by the NSF, the National Geographic Society, Vanderbilt University, and the Lapinski and O'Leary families. **Competing interests:** The authors declare that they have no competing interests. **Author contributions:** J.C.S. analyzed the textiles, recognized their significance, conceived the study, and coordinated the project. T.D.D. managed excavations at Huaca Prieta and supervised the radiocarbon dating of all strata used to date the textiles. J.W. (60%) and A.C. (40%) planned and performed the experiments and collected and analyzed all data associated with HPLC-PDA analysis. All authors contributed to the writing of the manuscript. The approximate individual author contribution breakdown is as follows: J.C.S. (31%), T.D.D. (30%), J.W. (30%), and A.C. (9%). **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 11 November 2015
Accepted 3 August 2016
Published 14 September 2016
10.1126/sciadv.1501623

Citation: J. C. Splitstoser, T. D. Dillehay, J. Wouters, A. Claro, Early pre-Hispanic use of indigo blue in Peru. *Sci. Adv.* **2**, e1501623 (2016).

Science Advances

Early pre-Hispanic use of indigo blue in Peru

Jeffrey C. Splitstoser, Tom D. Dillehay, Jan Wouters and Ana Claro

Sci Adv **2** (9), e1501623.
DOI: 10.1126/sciadv.1501623