Direct analysis in real time-mass spectroscopy for identification of red dye colourants in Paracas Necropolis Textiles

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Abstract The Paracas Necropolis on the south coast of Peru is renowned for the elaborate funerary bundles recovered from that dry sandy region. These bundles contained the remains of male leaders within the Paracas communities surrounded by multiple layers of plain cloth and garments with embroidered designs. The methods and materials used in dyeing the yarns used to embellish these ancient fabrics are still not well understood, and the research presented herein seeks to add to that body of knowledge. To investigate the sources of dye colourants in samples from Paracas Necropolis textiles, we applied direct analysis in real time–time-of-flight mass spectrometry (DART-MS). This new methodology has both advantages (analyses are rapid and require little or no sample preparation) and disadvantages (e.g. inability to identify intact carminic acid) compared to existing ones used in the analysis of dye colourants. Direct analysis in real time mass spectra were collected on chemical standards, botanical materials and comparative reference samples prepared with Relbunium roots and cochineal insects (Dactylopis sp.), and the results are compared to red fibres obtained from several different textiles within two different funerary bundles from the Museo Nacional de Arqueologia Antropologia e Historia in Lima, Peru. The results from the DART-MS analysis of these red fibres show that the compounds present are consistent with the presence of dye obtained from Relbunium species, and cochineal insects were not used to colour these fibres. 

Statement of significance DART-MS is an efficient and effective method to identify red colourant composition in small samples of fibres removed from archaeological textiles. Of significant note is the ability to differentiate isomers present in Relbunium and other anthraquinone dyes. Colourants in Relbunium are readily distinguished. Although a component of carminic acid from cochineal is less reliably positively identified by DART-MS, it is possible to use a simple extraction and ionization method to confirm or exclude the presence of carminic acid. Relbunium and cochineal are known to have been used in Paracas textiles. Not only does DART-MS aid in identification of colourants using small amounts of material and without significant sample preparation, the information can be used to distinguish fibres that were prepared differently thus indicating past knowledge of dyeing technology. The information can also aid in separating periods of artefact manufacture, and guide development of a conservation strategy.

Keywords Dyes, DART-MS, Paracas Necropolis, Textile, Anthraquinone

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Data availability The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are contained within the paper.
Introduction

The Paracas peninsula on the southern coast of Peru is renowned for the funerary bundles recovered from three sites in that dry sandy region. The bundles recovered from the Necropolis of Wari Kayan have been noted to be particularly elaborate, containing the remains of male leaders within the Paracas communities as well as multiple layers of textiles, garments and many other artefacts (Paul 1990a; Peters 2014, 110). While multiple chronologies have been proposed to frame the time period of this cultural tradition (e.g. Dwyer 1979), radiocarbon measurements of materials from the Paracas peninsula range in approximate date from the Early Horizon (400 BCE) through the Early Intermediate Period (600 AD) (Paul 1991a, 10). Between December 1927 and April 1928, Julio C. Tello removed some funerary bundles from the Necropolis of Wari Kayan in the Cerro Colorado region of the Paracas peninsula for curation at the Museo Nacional de Arqueología Antropología e Historia in Lima. The bundles range in size up to 1.5m high and 1.5m diameter and consist of numerous layers of plain woven undyed flat textiles employed to build bulk in the bundles and embroidered ponchos, tunics, loin cloths, headdresses and other garments, as well as tools, weapons and ritual ornaments (Paul 1990a; Peters, Cassman and Gustafsson 2007; Peters 2014, 110). Some of the textiles are well preserved due to the arid environment of the region though others display degradation. Because of the excellent condition of some of the fabrics, considerable research has been conducted describing their design and construction. Paul (1990a) describes the complete contents of two bundles (#378 and #310), and includes discussions of iconography; since no written words exist for the Paracas culture, the textiles serve to communicate about the plants and animals available locally as well as provide a view of the dress and adornment of the leaders. In a similar manner, Paul (1991b) describes the contents of another bundle (#89), which had been unwrapped in 1927 by Tello but which had not been reported until her examination in 1991. Peters (2014) explores specific types of garments from four bundles to discern social structure and exchange networks that are reflected and Peters, Cassman and Gustafsson review the contents of bundle #217 (Peters, Cassman and Gustafsson 2007). Peters (1991) describes the complex meanings of the embroidered plant and animal figures. “These figures surely allude to a complex of verbal and symbolic expressions (myths, proverbs, rituals, etc.)” (Peters 1991, p. 244). While many fabrics are made of cotton (Gossypium sp.), which likely was grown in nearby regions, the embroidery yarns are made from camelid fibres, either llama, alpaca or vicuña (Lama genus); these had to have been obtained from the mountainous regions of the country and their presence in the Paracas textiles indicate long distance exchange networks. Some camelid yarns also have cotton fibre mixed in (Jakes 1991).

The well preserved textiles display colours, which are still bright and attractive, unmarred by light exposure or by degradation within the archaeological context. Therefore, the methods and materials used in dyeing the embroidery yarns have been the subject of study. Paul (1990b) catalogues 240 different colours, grouped by 22 colour names (purple, red, pink, brown, dark brown, orange, gold, light gold, dark gold, olive, green light green, dark green, blue, light blue, turquoise, navy, dark purple, black, grey, beige and white). The artisans “shared principles of design, concepts of order, procedures of manufacture and a body of technical knowledge” (Paul 1990b, 20). Because the dyers shared dyeing recipes and produced a distinctive Paracas Necropolis palette unlike that found in textiles from other regions on the south coast, identification of colourants used in textiles could: (1) support their identification as belonging to Paracas Necropolis, (2) reveal the dyeing recipe that was employed, (3) aid in chronological assignment of the textile and (4) inform the conservator about the chemical composition of the material so that their preservation is insured.

Saltzman (1992) employed UV–visible spectroscopy of solutions of colourant extracted from fibre samples from different textiles from Paracas and found that the older textiles contain Relbunium while three younger textiles were dyed with cochineal obtained from the Dactylopius coccus insects. (Note: Botanists have argued whether plants labelled Relbunium constitute a distinct genus or if they belong to genus Galium because of the significant morphological similarities displayed by the two. De Toni and Mariath provide a recent review (De Toni and Mariath 2011) of this controversy and state their opinion that, based on multiple forms of evidence, the two are distinct genera. While the authors note that the controversy still prevails, in order to provide consistency with past literature related to textile dyes used prehistorically in South America, the term Relbunium is used within this manuscript rather than using the term Galium.) Colourants provided from Relbunium roots and cochineal insects create differing red shades, which are also influenced by the mordant used, but Saltzman (1992, 479) also argues that since the crushed cochineal insects are strongly red in colour, these insects are a more obvious choice as a colourant in comparison to the fine reddish roots of Relbunium; therefore, the predominant use of Relbunium must have some other unknown cause, perhaps related to a lack of access to cochineal or lack of the technology associated with cochineal dyeing (Rodríguez, Méndez and Neimeyer 2001). Fester (1954) and Antúnez de Mayolo (1989) both report that Al and Fe obtained from local materials were used as mordants in dyeing. Infrared and visible microanalysis confirmed the identification of Relbunium (Jakes, Katon and Martoglio 1991). Saito, Hayashi and Kojima (2003) identified the purpurin and pseudopurpurin components of Relbunium in three of...
four samples of Paracas textiles examined with HPLC; the fourth contained carminic acid attributable to cochineal. Microspectrofluorimetry of Paracas textiles indicated the presence of anthraquinone lakes formed by complexation of the colourants with aluminium (Claro et al. 2010).

**DART-MS for the identification of dyes in historic textile fibres**

The methods used in the past for identifying the organic dye colourants present in fibres from the Paracas Necropolis textiles have all involved spectroscopy, with or without chromatographic separation, on solutions extracted from the fibres. Mass spectrometry has several advantages over light spectroscopy methods, but has not, until now, been applied to the identification of dyes in these textiles. Direct analysis in real time (DART) ionisation coupled to time-of-flight mass spectrometry has been demonstrated as a rapid, simple method for the identification of dye colourants on cotton (Selvius DeRoo and Armitage 2011; Geiger, Armitage and Selvius DeRoo 2012) as well as on wool and silk fibres (Day, Selvius DeRoo and Armitage 2013). Most recently, DART-MS has shown that textile fibres from the Seip Mound group in Ohio were consistent with the use of anthraquinone dyes obtained from *Galium* species but did not show any evidence of benzisooquinoline alkaloids from bloodroot (*Sanguinaria canadensis*) (Armitage, Day and Jakes 2015).

**Goals of the Research**

This research was undertaken to investigate the sources of red dye colourants in samples from Paracas Necropolis textiles, using DART–time-of-flight mass spectrometry (DART-MS). Because DART-MS requires only minute samples with no sample preparation and a <5 min analysis time, the procedure is an ideal one for examination of archaeological textiles. It is important, however, to assemble an appropriate set of comparative materials and chemical standards. We show here that the flexibility and speed of the DART-MS method, yielding both parent ion and fragmentation patterns in a single analysis, provide both a significant insight into the nature of the anthraquinone dyes present in these samples and a way to differentiate *Relbunium* dyes from cochineal on the basis of their anthraquinone composition.

**Materials**

**Standards and comparative materials**

Chemical standards of alizarin, purpurin and carminic acid were obtained from various chemical suppliers (Table 1). Because they are not commercially available, standards of xanthopurpurin and rubiadin were synthesised in the laboratory for this study using a variation on a previously published method (Takano, Kondo and Nakatsubo 2006).

Table samples of *Relbunium* plant roots were obtained from two herbarium collections (Table 2). These roots were all run directly by DART-MS as described in the Procedure section. Because the samples of *Relbunium* root obtained were so small, no reference materials of dyed fibres could be prepared in our laboratory. However, samples of sheep and alpaca wool dyed with several species of *Relbunium* plants from the reference collection prepared by M. Saltzman (held at UCLA) were provided for analysis by DART-MS. In addition to the plant dyes, reference materials prepared from crushed cochineal insects from the Saltzman Collection as well as materials prepared in the Eastern Michigan University and Ohio State University laboratories with a variety of mordants on commercial undyed sheep and alpaca wool were also investigated.

**Historic materials**

Samples of archaeological fibres were obtained from several different textiles within two different funerary bundles in 1985 by Anne Paul from the materials housed in the *Museo Nacional de Antropología y Arqueología*; since that time, these materials have been stored in a climate controlled museum storage environment at Ohio State University. Only loose fibres and yarns found in close association with the stored

**Table 1** Chemical standards for direct analysis in real time-mass spectroscopy of anthraquinone dye colourants.

| Name                                           | Source          |
|------------------------------------------------|-----------------|
| Alizarin (1,2-dihydroxyanthraquinone)          | TCI America     |
| Purpurin (1,2,4-trihydroxyanthraquinone)       | TCI America     |
| Xanthopurpurin (1,3-dihydroxyanthraquinone)    | Synthesised by S. Augustin and T. L. Friebe |
| Rubiadin (1,3-dihydroxy-2-methylantraquinone)  | Synthesised by S. Augustin and T. L. Friebe |
| Carminic acid                                  | MP Biomedicals  |

**Table 2** *Relbunium* plant roots investigated by direct analysis in real time-mass spectroscopy (DART-MS).

| Genus and species      | Where collected/obtained or herbarium code |
|------------------------|-------------------------------------------|
| *Relbunium* hypocarpium| R. Busamann, Missouri Botanical Garden    |
| *Relbunium* richardianum| M. Tadesse, OSU Herbarium, #35349         |
| *Relbunium* unknown species | M. Tadesse, OSU Herbarium               |
textiles were collected; no samples were taken directly from the textiles themselves. Descriptions of the samples are provided in Table 3.

Methods

DART-MS analysis of standards and root samples

A small amount of each of the chemical standards either neat (commercial) or in ethyl acetate (synthesised) was collected on the closed end of a melting point capillary tube. For root samples, a small piece (2–3 mm long) was cut from the sample with a sterile scalpel blade. The standard sample on the tube or the root fragment in a pair of cleaned fine-tip tweezers was then introduced into the gap between the DART ionisation source (IonSense, Saugus, MA, USA) and Orifice 1 of the AccuTOF mass spectrometer (JEOL USA, Peabody, MA, USA). The DART gas temperature was 500°C for comparison with the dyed fibre samples. Standards were examined in negative ion mode consecutively applying four different voltages (−30, −60, −90, and −120 V) to Orifice 1. At −30 V, no fragmentation is observed and the primary peak is that of the \( \text{[M–H]}^- \) species, formed through the loss of one hydrogen atom. At increasingly negative voltages, collision-induced dissociation occurs, leading to increasing amounts of fragmentation. With Orifice 1 at −90 V, the collision-induced dissociation fragmentation pattern observed is similar to that obtained under electron impact (EI) conditions used in GC–MS analysis. While compounds of the same molecular formula yield identical spectra for the parent ion at −30 V, the fragmentation patterns at −90 V can be used to differentiate structural isomers. This method of in-source fragmentation using DART ionisation and the AccuTOF mass spectrometer has been validated for forensic identification of pharmaceuticals (Easter and Steiner 2014). The conditions of negative ion mode at 500°C have been shown to be ideal for the identification of anthraquinone dyes in proteinaceous fibres (Day, Selvius DeRoo and Armitage 2013).

Fibres were collected from the reference materials under the microscope using cleaned fine-tip reverse-action tweezers. The fibres were held in the tweezers, which were then placed within the gap between the DART ionisation source and the mass spectrometer inlet, where ionisation was carried out directly on the sample. The mass spectrometer settings were selected for maximum intensity in the range of interest (150–1000 Da), and were also carried out in orifice switching mode to examine the fragmentation patterns.

In examining the archaeological textile fibres, very small amounts of fibre were removed from the plastic bags or glass vials in which the samples had been stored. Once an amount of fibre that was clearly visible under magnification was collected in the fine-tipped tweezers, the material was introduced into the ion source under the conditions described above (500°C, negative ion mode, orifice switching from −30, −60, −90 and −120 V).

Results and Discussion

Chemical standards: Relbunium anthraquinones

Direct analysis in real time mass spectra for chemical standards of the four anthraquinones, which predominate in the composition of Relbunium species (Hofenk de Graaff 2004, pp. 130–132; Cardon 2007, pp. 162–164) were collected at −30 V (no fragmentation) and at −90 V (fragmentation due to collision induced dissociation); all of the DART spectra of the pure compounds are shown in Fig. 1. There are clear differences between the fragmentation patterns observed at −90 V for the spectra of alizarin and xanthopurpurin. Szostek et al. (2003) describe the peak at \( m/z \) 195 as deriving from the loss of CO\(_2\) (44 mass units), something that has been observed for the meta dihydroxy flavonoid compounds, and propose structures for each of the fragments in the xanthopurpurin mass spectrum. However, a small \( m/z \) 195 signal is observed in the commercial alizarin, either indicating an impurity or that there is another mechanism by which the loss of 44 mass units can occur. However, the ratio between the \( m/z \) 195 and 211 peaks for xanthopurpurin (0.95) is quite different from that observed for alizarin (0.12). The largest fragment ion observed for purpurin is at \( m/z \) 227, which is consistent with the loss of 28 mass units from the parent, as seen in published EI spectra of purpurin (e.g. in the NIST MS08 database (Various, 2008)). Synthesised rubiadin showed the most common fragment ion at −90 V to be \( m/z \) 225; no reference mass spectrum for rubiadin could be found in the literature for comparison.

Relbunium root samples

Figure 2 shows the −90 V spectra for the Relbunium root specimens, and Table 4 shows the distribution

| Identification number | Description |
|-----------------------|-------------|
| 421-39-03083 (83)     | Loose fibres, mantle (previously analysed by Saltzman 1992 and Jakes, Katon and Martoglio 1991) |
| 382-54 02846          | Loose threads, turban |
| 382-9 01671           | Embroidery thread, mantle |
| 421-39 03083          | Embroidery threads, turban |
| 382-54 02846          | Embroidery threads, turban |

1 Identification code: bundle number-specimen number-subspecimen number.
of anthraquinones observed in the $-30\text{V}$ spectra. The observed peak at $m/z$ 239.034 could be either xanthopurpurin or alizarin. While it is not possible to say from the $-90\text{V}$ DART spectra that alizarin is completely absent in the presence of xanthopurpurin, the pattern clearly indicates that xanthopurpurin is indeed present in all of the root samples, though mixed with alizarin in varying proportions. *R. hypocarpium* and *R. richardianum* both appear to contain significantly more xanthopurpurin than alizarin, based on the ratios of the $m/z$ 195, 210, and 211 peaks in the $-90\text{V}$ spectra. We confirmed that alizarin is present in quantities much lower than that of xanthopurpurin in both by use of HPLC-DAD analysis of EDTA/DMF extracts of the roots (unpublished results using methods from Manhita et al. (2014)). Based on the large $m/z$ 210 peak relative to the 195 and 211 peaks in DART, mass spectrum of the

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**Figure 1** Direct analysis in real time (DART) spectra for pure anthraquinone compounds.
unknown *Relbunium* species sample, alizarin is likely present in greater quantity relative to xanthopurpurin in this case, which was also confirmed by the HPLC analysis.

**Relbunium dye reference materials on fibres**

The Saltzman Collection of South American dye plants and insects on commercial sheep's wool and alpaca yarns were our sole source of reference for dyed fibres. The results of the DART-MS carried out at −30V on the yarns dyed with the three *Relbunium* species collected by Saltzman are shown in Table 5. The two main anthraquinones present in *R. hypocarpium* and the unknown species of *Relbunium* are xanthopurpurin (based on the ratios of the m/z 195 and 210 peaks in the -90V spectra) and purpurin. Dyes made from *Relbunium ciliatum* appear to contain significant quantities of alizarin in addition to xanthopurpurin, as the m/z 195/210 ratio is reversed for these samples. This was not confirmed by HPLC due to the small amount of material available. It is important to note that the ratios of the various anthraquinone colourants varied depending on the fibre and mordant composition, making it impossible to identify a specific species of *Relbunium* based solely on a qualitative DART-MS analysis.

**Table 4** Anthraquinones identified in *Relbunium* root samples in negative ion mode.

| Compound                              | Formula      | [M–H] ion | R. hypocarpium | R. richardianum | *Relbunium*, unknown sp. |
|---------------------------------------|--------------|-----------|----------------|----------------|--------------------------|
| Alizarin or xanthopurpurin            | C_{14}H_{8}O_{4} | 239.034   | ++ ++          | ++ ++          | +                        |
| Methoxymethylandraquinone             | C_{16}H_{12}O_{3} | 251.074   | nd             | tr             | Nd                       |
| Rubiadin or alizarin methyl ether     | C_{15}H_{10}O_{4} | 253.052   | +              | ++++           | ++                       |
| Purpurin                              | C_{14}H_{8}O_{4} | 255.031   | ++++           | ++++           | +                        |
| Xanthopurpurin dimethyl ether         | C_{16}H_{12}O_{4} | 267.066   | nd             | nd             | Nd                       |
| Lucidin                               | C_{17}H_{12}O_{6} | 269.048   | +              | ++             | +                        |
| Munjistin                             | C_{16}H_{8}O_{5} | 283.024   | tr             | +              | Nd                       |
| Pseudopurpurin                        | C_{16}H_{10}O_{7} | 299.019   | nd             | nd             | Tr                       |

Key: nd: not detected; each “+”: a multiple of 20% of the observed base peak; trace (tr): < 10% relative abundance.
Dutra Moresi and Wouters (1997) state that the complete lack of alizarin is a mark of dyes prepared from the roots of the *Relbunium* genus. This further demonstrates the difficulty of comparing previously published work on *Relbunium* dyes carried out with liquid chromatographic methods on extracts of roots and dyed textiles to direct analysis of roots and fibres with DART-MS. The process of extraction removes only some of the dye compounds from the material, and the inherent lack of selectivity of UV–visible spectroscopic detection must also be accounted for in making these comparisons. It is therefore critical that data-bases of reference materials that have been analysed by use of DART-MS be created and expanded in order to make accurate comparisons, rather than relying on comparing two methods of significantly differing sensitivity and preparation methods.

**Carminic acid and cochineal standards and reference materials**

Pure carminic acid analysed by DART-MS at 500°C in negative ion mode shows no peak for the expected [M–H]− ion at m/z 491.083. Rather, a low intensity signal for the aglycone of carminic acid (also called kermesic acid) is observed at m/z 329.029. Though the molecular mass of carminic acid is within the range of the DART ionisation method, the compound is not volatile enough to be desorbed by heat alone, probably due to strong hydrogen bonding of the glucose moiety. Adding a small amount of 88% formic acid aids in hydrolysis of the glycosidic linkage, thereby yielding an enhanced signal for the anthraquinone aglycone, as has been described previously (Day, Selvius DeRoo and Armitage 2013).

Even the formic acid treatment appears to be insufficient in some cases for identifying cochineal on fibres, either because of the mordant or due to the low concentration of carminic acid present. For comparison, fibre samples were extracted using a mixture of EDTA and DMF (Manhita et al. 2011) and analysed in negative ion mode by electrospray ionisation mass spectrometry (ESI−). Samples were infused directly into the JEOL ESI source using a syringe pump; they were not subjected to HPLC for separation prior to MS. A comparison of the DART-MS and ESI− results is shown in Table 6. While carminic acid is readily detected by ESI− on the extract from the fibres, DART-MS is less reliable for identifying the colourants characteristic of cochineal directly from the fibres.

**Table 5 Anthraquinones identified in *Relbunium*-dyed yarns from the Saltzman reference collection in negative ion mode.**

| Compound                  | Formula          | [M–H]− ion | Wool | Alpaca | Wool | Alpaca | Wool | Alpaca |
|---------------------------|------------------|------------|------|--------|------|--------|------|--------|
| alizarin or xanthopurpurin| C14H8O4          | 239.034    | +    | tr     | tr   | +      | tr   | tr     |
| methoxymethylanthraquinone| C14H12O3         | 251.074    | nd   | nd     | nd   | nd     | nd   | nd     |
| Rubiadin or alizarin methyl ether| C15H10O4       | 253.052    | tr   | tr     | tr   | tr     | tr   | tr     |
| Purpurin                  | C14H8O4          | 255.031    | ++   | ++     | ++   | ++     | ++   | ++     |
| Lucidin                   | C15H10O5         | 269.048    | tr   | tr     | tr   | tr     | tr   | tr     |
| Munjistin                 | C15H10O5         | 283.024    | tr   | tr     | tr   | tr     | tr   | tr     |
| Pseudopurpurin            | C16H12O7         | 299.019    | tr   | tr     | tr   | nd     | nd   | nd     |

Key: nd: not detected; each “+”: a multiple of 20% of the observed base peak; trace (tr): <10% relative abundance. N: no mordant; A: alum mordant.

**Table 6 Comparison of direct analysis in real-time mass spectrometry (DART-MS) and electrospray ionisation (ESI−) for identification of cochineal dye colourants in fibres.**

| Fibre          | Mordant | Source | DART-MS w/formic acid | ESI− on extract |
|----------------|---------|--------|------------------------|-----------------|
| Sheep wool     | None    | Saltzman| KA only                | CA              |
| Alpaca wool    | None    | Saltzman| None                   | CA              |
| Sheep wool     | Alum    | Saltzman| None                   | CA              |
| Alpaca wool    | Alum    | Saltzman| FK only                | CA              |
| Alpaca wool    | None    | EMU     | None                   | CA (trace)      |
| Alpaca wool    | Alum    | EMU     | KA, FK (traces)        | CA, KA (traces) |
| Alpaca wool    | Iron    | EMU     | KA, FK (traces)        | CA, KA (traces) |
| Sheep wool     | None    | OSU     | KA                     | n/a             |
| Sheep wool     | Alum    | OSU     | KA (trace)             | n/a             |
| Sheep wool     | Copper  | OSU     | KA, FK (traces)        | n/a             |
| Sheep wool     | Iron    | OSU     | KA, FK (traces)        | n/a             |
| Sheep wool     | Tin     | OSU     | KA, FK; CA (trace)     | n/a             |

CA: carminic acid; KA: kermesic acid (aglycone of CA); FK: flavokermesic acid.
Archaeological samples

The Paracas Necropolis samples were studied by DART-MS in negative ion mode, both with and without the addition of formic acid. The results of the DART-MS analysis at $-30V$ are shown in Table 7. Purpurin and presumably xanthopurpurin are the primary anthraquinones present in all of the Paracas samples. Because the signal intensities were quite low in the $-30V$ spectra, the DART-MS spectra obtained at $-90V$ were of insufficient intensity to compare the fragmentation patterns that would aid in clarifying the source of the $m/z$ 239.034 peak. One of the Paracas samples (421-39 03083 [IV]) provided for analysis in this study had previously been characterised by UV, IR, and visible microspectroscopies and determined to contain dye colourants characteristic of *Relbunium* (Saltzman 1978; Jakes, Katon and Martoglio 1991). Analysis of this same sample by DART-MS showed that purpurin, xanthopurpurin, and munjistin were all present, as would be expected from the previous identification of *Relbunium* in this material. These anthraquinone compounds were also identified in all of the other red samples, as was pseudopurpurin in two of the samples. The results from the DART-MS analysis of these red fibres show that the compounds present are consistent with the presence of dye from *Relbunium* plant roots.

No evidence of the cochineal colourants was observed by DART-MS in any of the formic acid-treated Paracas Necropolis textile fibres. Because the small size of most of the samples precluded extracting them, only the largest was subjected to extraction and ESI. Neither was carminic acid observed in the ESI mass spectrum nor were there any of the other characteristic compounds for cochineal. This is consistent with Saltzman’s analyses that the majority of Paracas Necropolis period textiles do not show evidence of having been dyed with cochineal insects: only three of 141 samples examined by Saltzman could be attributed to cochineal, while the remainder were coloured with *Relbunium* (Saltzman 1992).

The two bundles from which the specimens employed in this work were obtained were categorised chronologically by Paul (1990a, 60) according to the stylistic sequence proposed by Dwyer (1979). While both belong to the Early Intermediate period (200–600 AD), Bundle 421 was placed as part of an earlier phase (1A), while Bundle 382 was categorised as part of a later phase (1B). Since there is a trend towards increased use of cochineal in Andean textiles (Fester 1954; Saltzman 1992), consistent use of *Relbunium* in the examples studied supports their categorisation in this earlier period.

Conclusions

It has been shown that DART-MS is an efficient and effective avenue to identify red colourant composition in small samples of fibres removed from archaeological textiles. Of significant note is the ability to use in-source collision-induced dissociation to differentiate...
isomers present in Relbunium and other anthraquinone dyes. The procedure can readily distinguish the Relbunium dye colourants in both reference and archaeological fibres. The aglycone of carminic acid from cochenille is less reliably positively identified by DART-MS, probably related to the interaction of the dye, fibre and mordant. Much further work is needed to better identify cochenile colourants in fibres by DART-MS. When the cochenile colourants are not observed by DART-MS, it is still possible to use a simple extraction and electrospray ionisation without separation to confirm or exclude the presence of carminic acid. These two red colourants are known to have been used in textiles from the Paracas peninsula, and DART-MS can be used to screen for these colourants rapidly and without significant sample preparation. Not only does this work aid in identification of colourants using very small amounts of material, the information can be used to distinguish fibres that were prepared differently, perhaps separating periods of manufacture, as suggested by Saltzman (1992). The information also is helpful in guiding a conservator in developing a conservation strategy. Future work will be dedicated to developing a comprehensive database of DART mass spectra obtained on a variety of plant dyes, for comparative studies and to determine if the method can be used to distinguish between various plants of the Rubiaceae family.

Conflicts of Interest
The authors declare that they have no conflict of interest.

Author biographies
Ruth Ann Armitage is a professor of chemistry at Eastern Michigan University, Ypsilanti, MI. Her research has focused on developing analytical methods to study archaeological materials like rock paintings and textiles to better understand human behavior in the past.

Kathryn Jakes is professor emeritus at Ohio State University, Columbus and an independent scholar in archaeological textiles. Her research throughout her career has been uncovering clues to the past through analysis of archaeological and historical textile materials.

Calvin J. Day was an undergraduate research student in the Armitage lab at Eastern Michigan University. He graduated with a Bachelor’s degree in Chemistry from EMU in August 2014 and is currently employed by Reliable Analysis in southeastern Michigan.

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References
Antúnez de Mayolo, Kay K. 1989. “Peruvian Natural Dye Plants.” Economic Botany 43 (2): 181–191.

Armitage, Ruth Ann, Calvin J. Day, and Kathryn A. Jakes. 2015. “Identification of anthraquinone dye colourants in red fibres from an Ohio Hopewell mound site by direct analysis in real time mass spectrometry.” Science and Technology in Archaeological Research 1 (2), STAR2015122054892315Y.0000000009

Cardon, Dominique. 2007. Natural Dyes: Sources, Tradition, Technology and Science. London: Archetype.

Claro, Ana, Maria J. Melo, J. Sérico Seixas de Melo, Klaas Jan van den Berg, Aviva Burnstock, Meredith Montague, and Richard Newman. 2010. “Identification of Red Colorants in van Gogh Paintings and Ancient Andean Textiles by Microspectrofluorimetry.” Journal of Cultural Heritage 11 (1): 27–34.

Day, Calvin J., Cathy Selvius DeRoo, and Ruth Ann Armitage. 2013. “Developing Direct Analysis in Real Time Time-of-Flight Mass Spectrometric Methods for Identification of Organic Dyes in Historic Wool Textiles.” In Archaeological Chemistry VIII, edited by R. A. Armitage and J. H. Burton, 69–85. Washington, DC: American Chemical Society.

De Toni, Karen L. G., and Jorge E. A. Mariath. 2011. “Developmental Anatomy and Morphology of the Flowers and Fruits of Species from Galium and Relbunium (Rubiaceae, Rubiaceae).” Annals of the Missouri Botanical Garden 98 (2): 206–225.

Dutra Moreira, Claudia Maria, and Jan Wouters. 1997. “HPLC Analysis of Extracts, Dyeyings and Lakes, Prepared with 21 Species of Relbunium.” In Dyestuffs and Lakes, edited by A. Paul, 22–39. Iowa City: University of Iowa Press.
Jakes, Kathryn A., Jack E. Katon, and Pamela A. Martoglio. 1991. “Identification of Dyes and Characterization of Fibers by Infrared and Visible Microspectroscopy: Application to Paracas Textiles.” In Archaeometry 30, edited by G. A. Wagner and E. Pernicka. Basel: Birkhäuser: 305–315.

Manhita, Ana, Lieve Balcaen, Frank Vanhaecke, Teresa Ferreira, António Candeias, and Cristina Barrocas Dias. 2011. “Extracting Natural Dyes from wool – An Evaluation of Extraction Methods.” Analytical and Bioanalytical Chemistry 400: 1501–1514.

Paul, Anne. 1990a. Paracas Ritual Attire: Symbols of Authority in Ancient Peru. Norman, OK: University of Oklahoma Press.

Paul, Anne. 1990b. “The Use of Color in Paracas Necropolis Fabrics: What Does it Reveal About the Organization of Dyeing, Designing and Society?” National Geographic Research 6 (1): 7–21.

Paul, Anne, ed. 1991a. Paracas Art and Architecture. Iowa City: University of Iowa Press.

Saltzman, Max. 1978. “The identification of dyes in archaeological and ethnographic textiles.” In Archaeological Chemistry II, edited by G. Carter, 172–185. Washington, DC: American Chemical Society.

Saltzman, Max. 1992. “Identifying Dyes in Textiles.” American Scientist 80 (5): 474–481.

Selvius DeRoo, Cathy, and Ruth Ann Armitage. 2011. “Direct Identification of Dyes in Textiles by Direct Analysis in Real Time-Time of Flight Mass Spectrometry.” Analytical Chemistry 83 (18): 6924–6928.

Saito, Masako, Akiko Hayashi, and Mariko Kojima. 2003. “Identification of Six Natural Red Dyes by High-Performance Liquid Chromatography.” In Dyes in History and Archaeology 19, edited by J. Kirby, 79–87. London: Archetype.

Peters, Anne H. 2014. “Paracas Necropolis: Communities of Textile Production, Exchange Networks and Social Boundaries in the Central Andes, 150 BC to AD 250.” In Textiles. Technical Practice and Power in the Andes, edited by D. Arnold and P. Dransart, 109–139. London: Archetype.

Rodríguez, Luis C., Marco A. Méndez, and Hermann N. Neimeyer. 2001. “Direction of Dispersion of Cochineal (Dactylopius coccus Costa) Within the Americas.” Antiquity 75: 73–77.

Saltzman, Max. 1992. “Identifying Dyes in Textiles.” American Scientist 80 (5): 474–481.