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# Multi-element analysis using inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission spectroscopy for provenancing of animals at the continental scale

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ABSTRACT

Chemical signatures within the environment vary between regions as a result of climatological, geochemical and anthropogenic influences. These variations are incorporated into the region's geology, soils, water and vegetation; ultimately making their way through the food chain to higher level organisms. Because the variation in chemical signatures between areas is significant, a specific knowledge of differences in elemental distribution patterns between, and within populations, could prove beneficial for provenancing animals or animal related products when applied to indigenous and feral faunal populations. The domestic pig (*Sus scrofa domestica*) was used as an investigative model to determine the feasibility of using a chemical traceability method for the provenance determination of animal tissue. Samples of pig muscle, tongue, stomach, heart, liver and kidney were collected from known farming areas around Australia. Samples were digested in 1:3 $H_2O_2$:$HNO_3$ and their elemental composition determined using solution based Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Pigs from different growing regions in Australia could be distinguished based on the chemical signature of each individual tissue type. Discrimination was possible at a region, state and population level. This investigation demonstrates the potential for multi-element analysis of low genetic variation native and feral species of forensic relevance.



## 1. Introduction

The ability to trace the source of recovered plants and animals, or their by-products, is essential to the detection and prosecution of wildlife related crimes. Where animals are hunted or traded, a crime must first be established by determining if the specimen was harvested from a managed legal population or from an illegal source [1]. With managed populations, a need also exists to ensure illegal translocation of wildlife is not occurring [2]. In other circumstances, where a crime is known to have been committed, such as the smuggling of CITES listed species, the ability to establish the known region of origin of the evidence may play a pivotal role in determining hotspots for criminal activity, thus providing intelligence to law enforcement agencies [3]. The low penalties and degree of risk associated with wildlife crime, coupled with potentially huge profits exceeding those of drug and arms trafficking weight for weight, has lead to wildlife trafficking being a domain of organised crime syndicates [4]. This has resulted in concomitant increases in difficulty in both finding and identifying evidence as the professionalism increases [1]. The ability to trace multiple seizures back to a single geographic region of origin can therefore play a large role in stopping such operations at the source and interrupting the overharvesting of at-risk populations [3].

Commonly, genetic methods of population assignment are used to assess the geographic origin of unknown animal and plant material in forensics cases. A multitude of analytical techniques have been developed to facilitate the development of a genetic profile for assignment testing [1]. Depending on the species of interest, assignment or exclusion to a distinct population or origin is then conducted based the known database [2]. For more mobile

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species, and those in which the database is not complete, assignment is made to a broad region using geographic maps of allele frequency distributions [3]. However, the degree to which genetic modelling techniques can match an individual to a population is technique dependant and not all genetic tools provide the same resolution [1].

If populations of a given species exist in clearly defined, genetically distinct groups, genetic characterisation and identification to an area of distribution can be very accurate using genetic assignment tools [1]. With the use of geographic genetic modelling techniques, such as those performed by Wasser et al. [3] for ivory seizures, the ability to link a seized wildlife product back to a geographic region without reference samples is further increasing the usefulness of genetic profiling in wildlife provenance investigations. However, when the populations do not have distinct boundaries or genetic divergence between populations is low, the use of genetic techniques for the geographic assignment becomes difficult [1]. In such incidences, implementation of chemical traceability techniques, in conjunction with genetic analysis, could greatly advantage the ongoing investigation.

Chemical methods of geographic assessment are less common in wildlife forensic investigations than genetic techniques. However, considerable work on ecological research has exploited chemical profiling as a means to trace the geographic association of highly mobile migratory animals, facilitating the development of the technique for wildlife forensics [5]. Further, chemical markers are utilised in standard forensic investigations with stable isotopes used to assess the geographic origin of a multitude of sample types from heroine [6] to unidentified homicide victims [7]. In a wildlife crime context Kelly et al. [8] demonstrated the potential to distinguish captive breed versus illegally caught songbirds using stable hydrogen isotopic analysis. Using isotopic precipitation maps these authors were able to identify possible geographic regions of origin without the need to have sampled the chemical signature of the different populations; therefore, allowing for predictive assignment of wildlife products to a geographic region of origin [5]. However such predictive assignments are heavily reliant on populations coming from chemically distinct geographic regions [8].

Multi-element distribution pattern identification is a new approach in the area of forensic provenancing. The application of this technology has its roots in gold and diamond provenancing [9,10] with considerable application into the provenancing of scene of crime evidence [11]. While trace element analysis does not facilitate the use of predictive maps, it can distinguish known populations at smaller spatial scales than isotopic analysis and over the last five years the technology has been applied to a greater extent for provenancing food products [12–16].

This paper details the use of multi-element analysis and the discriminatory power of inter-element association pattern recognition for population distribution assessment of the domestic pig (*Sus scrofa domestica*). The domestic pig was used as a model to demonstrate the potential benefit of the technology to the field of wildlife forensics.

## 2. Materials and methods

### 2.1. Reagents and samples

Sampling occurred on the slaughter floor from 127 pigs, from 24 different farms around Australia (Fig. 1). To ensure consistency of sampling, tissues were sub-sampled from the belly (muscle), transversalis muscle (tongue), pyloris (stomach), left ventricular wall (heart), left lateral lobe (liver) and the renal cortex (kidney). Pigs consisted of female, intact males and immune-castrated males. Post-sampling, tissues were frozen at −9 °C until preparation.

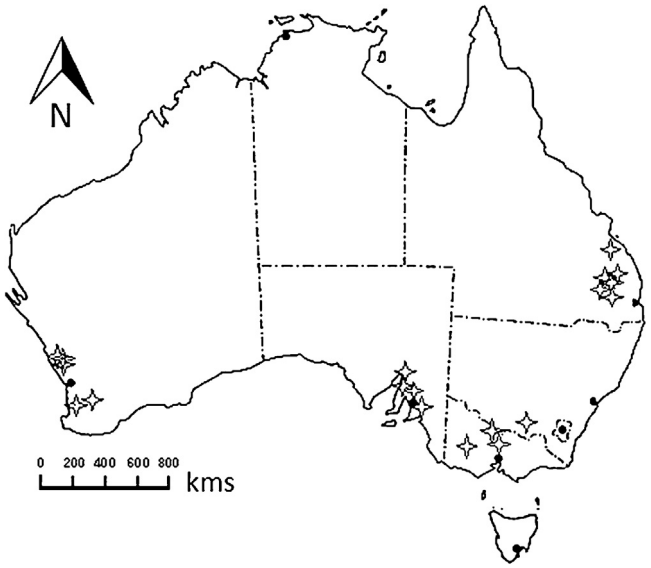


**Fig. 1.** Distribution of Australian pig farms sampled between 2010 and 2012 (open stars) and their proximity to major cities (solid circles).

Sampled pigs came from commercial farms across Australia and were subjected to farm specific practices including nutrient supplementation, feeding of commercial fodder and restricted access to the outdoor environment. As pigs were collected at slaughter, feeding regimes for subject animals is unknown.

Laboratory grade $HNO_3$ and 30% $H_2O_2$ were obtained through Univar from Ajax Finechem Pty. Ltd. Nitric acid was distilled using sub-boiling quartz stills (Quarzglas Komponenten und Services QCS GmbH, Germany). The final redistilled acid and hydrogen peroxide were quality control checked for the concentration of all analytes used in this study using ICP-MS.

### 2.2. Sample preparation

A 3:1 $HNO_3$:$H_2O_2$ acid digest adapted from that described by Watling et al. [16] was used to prepare all samples for solution based analysis. Approximately 2 g wet weight of sample, 0.5 g 1577c CRM and 0.5 g of an internal muscle standard were cold digested for 48 h in 6 mL of $HNO_3$. After the initial oxidation, 2 mL of $H_2O_2$ were added and the samples refluxed on a water bath for 24 h. A further 2 mL of $H_2O_2$ were added and the samples were reflux for 2 h. Solutions were brought down to 2 mL before being made to volume (30 mL) using 18 MegΩ deionised water. Three blanks, three CRM's, and three in-house standards were treated in the same manner for each analytical run. Fifteen percent of samples were analysed in duplicate and a minimum of three cross-over samples (randomly selected samples from a previous data set) per tissue type were incorporated into each batch. The replicate samples were analysed at the end of each analytical run to determine reproducibility of analytical data. Digested samples were diluted in 2% redistilled $HNO_3$ containing 2 ppb of Rh and Ir as internal standards. Internal standards were used to monitor drift during analysis. National Institute of Standards and Technology (NIST) bovine liver standard CRM 1577c was used to measure analytical accuracy. Mean observed values for the NIST standard are given in Table S1. An in-house freeze dried muscle standard was used to check batch to batch corrections.

### 2.3. Sample analysis and data interpretation

An ICP-AES Thermo Scientific iCAP 6500 Duo and an ICP-MS Agilent 7700× were used throughout this study. Both systems